DATA EVALUATION RECORD

9/1/1999

PIRIMICARB

Study Type: 82-7: Subchronic Neurotoxicity Study - Rats
Work Assignment No. 3-49A (MRID 44233101)

Prepared for

Health Effects Division
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U.S. Environmental Protection Agency
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Oral Gavage Study in Rats
OPPTS Number: 870.6200 OPP Guideline Number: §82-7

DP BARCODE: D240068 SUBMISSION CODE: S531972
P.C. CODE: 0531972 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Pirimicarb (97.6% a.i.)

SYNONYMS: 5,6-Dimethyl-2-dimethylamino-4-dimethylcarbamoyl-oxy-pyrimidine; 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (IUPAC); 2-(dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate (CA); PP062

CITATION: Horner, S.A. (1996) Pirimicarb: Subchronic neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ. Laboratory Project No. PR1048. November 27, 1996. MRID 44233101. Unpublished.

SPONSOR: Zeneca Ag Products, 1800 Concord Pike, Wilmington, DE 19850

EXECUTIVE SUMMARY:

In a subchronic neurotoxicity study (MRID 44233101), pirimicarb (97.6% a.i.) was administered to Alpk:AP$_f$SD (Wistar-derived) rats (12/sex/dose) at dietary concentrations of 0, 75, 250 or 1000 ppm (0, 5.6, 19.2 or 77.1 mg/kg/day for males; 0, 6.6, 21.8 or 84.4 mg/kg/day for females) for 13 weeks. All rats were evaluated by functional observation battery (FOB) and motor activity testing prior to treatment and during weeks 5, 9, and 14. Six rats/sex/group were evaluated for neuropathology and the remaining 6 rats/sex/group were evaluated for cholinesterase activities (plasma, erythrocyte, and brain) at the end of the study.

No rats died during the study. No treatment-related neurotoxicological effects or differences in FOB assessment or motor activity results were observed. The 1000 ppm treatment groups had mean final body weights 8-9% lower than the control body weights. Both sexes consumed less food than the controls; mean weekly food consumption values were ≤ 6% lower for males and 7-20% lower for females compared to the control values. The 250 ppm treatment groups had mean body weights ≤5-6% lower than the controls that were significantly ($p \leq 0.05$)

decreased for males at weeks 7-14, and for females at weeks 9 and 12-14. Food consumption was not affected by treatment. The 75 ppm treatment groups appeared to be unaffected by treatment. For all treatment groups, no treatment-related differences in absolute or relative brain weights or in brain, erythrocyte, plasma cholinesterase or neuropathy target esterase activities were observed. No treatment-related gross pathological abnormalities were observed in any treatment group. No macroscopic or microscopic abnormalities in nervous system tissues from 1000 ppm group rats were observed. The positive control data for the laboratory was considered adequate to assure that the laboratory could interpret the data. **No neurotoxicological effects were observed at 1000 ppm, the highest dose tested. The toxicological LOAEL for this study is 250 ppm (19.2 mg/kg/day), based on decreased body weights and food consumption in both sexes. The toxicological NOAEL is 75 ppm (5.6 mg/kg/day), based on the lack of effects on body weights and food consumption noted in this study, in conjunction with a lack of gross and/or histopathological findings in a subchronic toxicity study (MRID 44233103) reviewed in conjunction with this study.**

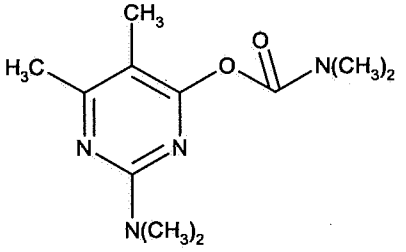
This study is classified **acceptable(guideline)** and satisfies the guideline requirement for a subchronic neurotoxicity study in rodents (§82-7).

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Quality Assurance, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Pirimicarb
   Description: White solid
   Batch No.: P16
   CTL Reference No.: Y00032/026
   Purity: 97.6% a.i.
   Stability of compound: It was stated that the test substance was used within the expiration date and that its stability was confirmed by re-analysis after dosing had ended. Stability data were not provided.
   CAS #: 23103-98-2
   Structure:



2. Vehicle and/or positive control: No vehicle
Positive control or validation studies (see TXR #011013, MRID43013301 through -05) were submitted to support previously submitted rat neurotoxicity studies and are considered acceptable to satisfy the neurotoxicity guideline requirement for demonstration of proficiency of the testing laboratory in evaluating neurobehavioral effects. Appropriate responses were identified for each of the positive control chemicals tested. The study demonstrated the capability of the testing laboratory, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ, to conduct adequate functional operational battery, grip strength, motor activity testing and neuropathology evaluations.

3. Test animals: Species: Rat
   Strain: Alpk:AP$_f$SD (Wistar-derived)
   Age at study initiation: ≥42 days old
   Weight at study initiation: Males, 178-212 g; females, 122-146 g
   Source: Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

Housing: Housed in cages in groups of four
Diet: CT1 diet (Special Diet Services Limited, Stepfield, Witham, Essex, UK), ad libitum
Water: Tap water, ad libitum
Environmental conditions:
Temperature: 21 ± 2 C
Humidity: 55 ± 15%
Air changes: ≥15/hour
Photoperiod: 12-Hour light/dark cycle
Acclimation period: Approximately 2 weeks

B. STUDY DESIGN

1. In-life dates - 3/12/96 to 6/14/96

2. Animal assignment

Animals considered to be healthy (including responding to a pre-randomization tail-flick test) and not at the extremes of the body weight range of the laboratory stock were selected for use in the study. Healthy animals were randomly assigned to the test groups in Table 1. The test groups were arranged on two racks in six single-sex replicates (randomized blocks). Each replicate consisted of four cages, one cage per treatment group.

Table 1. Study design.[a]

Test Group	Dose to animal (ppm)	Animals Assigned	
		Male	Female
1 Control	0	12	12
2 Low	75	12	12
3 Mid	250	12	12
4 High	1000	12	12

[a] The rationale for dose selection was not provided.
[b] Neurobehavioral evaluation was performed on all rats in each treatment group. Neuropathological examinations were performed on 6 rats/sex/dose.

3. Treatment preparation

The test diets were prepared in 30-kg batches from a premix prepared by triturating the appropriate amount of pirimicarb with 500 g of milled diet. The premix was added to 29.5 kg of CT1 diet and mixed in a blender for 6 minutes. The amounts of pirimicarb added to 30 kg of diet to obtain concentrations of 75, 250, and 750 ppm were 2.3, 7.7, and 30.7 g, respectively. The prepared diets were stored in glass jars at room temperature until use.

The homogeneity of treated diet was determined by analyzing samples from the 75 and 1000 ppm treatment preparations. Homogeneity samples were collected from different locations within the sample containers. The stability of pirimicarb in the diet was determined in 75 and 1000 ppm treatment preparations stored at room temperature for 43 days. The concentration of pirimicarb was determined in treated diet prepared before study initiation (3/1/96) and during treatment (4/24/96).

Results:
Homogeneity analysis:
75 ppm: 96.3-99.2% of nominal (mean 98.1%)
1000 ppm: 99.4-103% of nominal (mean 101.6%)
Stability analysis (43 days):
75 ppm: 110.9% of nominal
1000 ppm: 104.0% of nominal
Concentration analysis (mean values):
(prepared 3/1/96):
75 ppm: 97.6% of nominal
250 ppm: 99.2% of nominal
750 ppm: 100.6% of nominal
(prepared 4/24/96):
75 ppm: 101.7% of nominal
250 ppm: 99.6% of nominal
750 ppm: 99.3% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dose to the animals was acceptable.

4. Statistics

Weekly food consumption and food utilization during weeks 1-4, 5-8, 9-13, and 1-13, motor activity measurements, time-to-tail flick, landing foot splay, grip strength, brain weight, length, and width, and blood and brain cholinesterase activity were analyzed for each sex using analysis of variance. Body weights adjusted for initial body weight, and brain weight, length, and width adjusted for final body weight were analyzed for

each sex using analysis of covariance. Analysis of variance and covariance allowed for the replicate structure of the study design. For each parameter, least-squares means were calculated for each test group using SAS. Differences from control were statistically tested 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. Significance was determined at the 5 and 1% levels.

C. METHODS

1. Observations

Prior to study initiation, all animals were examined to ensure that they were physically normal and exhibited normal activity. Clinical condition or behavior were observed daily for changes. Detailed clinical examinations, including the finding of no abnormalities detected, were recorded weekly.

2. Body weight

Body weights of all rats were recorded immediately before feeding at study commencement, and on the same day, when possible, of each subsequent week until study termination.

3. Food consumption and compound intake

Food consumption by all rats within each cage was recorded continuously, and was calculated as the weekly mean (g food/rat/day) for each cage. The mean food efficiency value for each cage of rats was calculated as total body weight gain for all rats within a cage per 100 g of food consumed. Compound intake (mg/kg body weight) was calculated separately for each cage as:

$$\frac{\text{nominal concentration (ppm) x cage food consumption for week x}}{\text{estimated cage mean body weight at the middle of week x.}}$$

4. Neurobehavioral Studies

Functional observational battery (FOB) and motor activity testing were performed on all animals during the week prior to initiation of treatment and during weeks 5, 8, and 14 of treatment.

Functional Observational Battery - The major groups of FOB observations/measurements are listed below. Detailed clinical observations during which the animal was removed from its cage and physically examined for general health status, and quantitative assessments of landing foot splay, muscle weakness

(fore- and hindlimb grip strength), and sensory perception (tail-flick test) were made at weeks -1, 5, 9, and 14. Observations were made by one observer who was "blind" with respect to the animal's treatment, and were recorded on a computer system by personnel not directly involved in the clinical observations. The presence and/or absence of all listed observations was recorded and the degree of condition noted (slight, moderate or extreme). Clinical observations included, but were not limited to, observations/measurements listed below. Positive control data were not included in this submission; however, acceptable positive control data for morphine sulfate, chlordiazepoxide hydrochloride, amphetamine sulfate, chlorpromazine, trimethyltin chloride, and acrylamide from previous studies (MRIDs 43013301, 43013302, 43013303, 43013304, and 43013305) were available for comparison purposes.

FOB ASSESSMENT OBSERVATIONS/MEASUREMENTS

Signs of autonomic function such as lachrymation, salivation, piloerection, exophthalmus, urinary incontinence, diarrhea, pupillary response to light and ptosis.

Convulsions, tremors or abnormal motor movements in the home cage and open arena.

Arousal level or state of alertness in the open arena.

Posture and gait abnormalities in the home cage and open arena.

Response to sudden sound.

Unusual or abnormal behaviors, stereotypy, emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose or mouth, and other observations.

Motor Activity - Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested one week prior to study initiation and at weeks 5, 9, and 14 of the exposure period. Each observation period was divided into ten scans of five-minute durations. Treatment groups were counter-balanced across test times and devices. When the trials were repeated, each animal was returned to the same activity monitor at approximately the same time of the day. Motor activity was assessed in a separate room to minimize disturbances.

5. Cholinesterase Measurements

Six rats/sex/group designated for cholinesterase measurements were killed by exsanguination under terminal anaesthesia induced by halothane Ph.Eur. vapor. Blood samples were collected during exsanguination for determination of plasma and

erythrocyte activity. The brains of these animals were removed. The left half of each brain was used to determine brain enzyme activity. The right half was used to determine neuropathy target esterase activity.

6. Sacrifice and Pathology

At the end of the study, 6 rats/sex/group were deeply anesthetized with barbiturate i.p. and killed by perfusion fixation with modified Karnovsky's fixative. The tissues listed below were removed and brain weight, length, and width were recorded. The tissues were microscopically examined. Neuropathological examinations were performed on the control and 1000 ppm groups only. All sections were examined by light microscopy.

BRAIN Cerebral cortex[a] Hippocampus[a] Cerebellum[a] Pons[a] Medulla oblongata[a] SPINAL CORD Cervical (C3-C6)[a] Lumbar (L1-L4)[a]	SPINAL NERVE ROOT FIBER AND GANGLION Dorsal root ganglia with spinal roots (C3-C6 and L1-L4) Gasserian ganglia from trigeminal nerve[c] Gastrocnemius muscle[a] PERIPHERAL NERVES Sciatic (bilateral)[c] Tibial (bilateral)[a] Sural (bilateral)[a]

[a] Transverse sections of these tissues were evaluated. The brain was examined at 7 levels.
[b] Longitudinal sections of these tissues were evaluated.
[c] Transverse and longitudinal sections of these tissues were evaluated.

The brain and gastrocnemius muscle were embedded in paraffin wax, cut, and stained with H & E stain. Transverse sections of the vertebral column containing samples from the lumbar and cervical regions of the spinal cord, with dorsal root ganglia and spinal roots attached, were decalcified, embedded in paraffin wax, cut and stained with H & E stain. The remaining tissues and samples of the spinal cord and peripheral nerves were embedded in Araldite, cut into semi-thin sections, and stained with toluidine blue.

II. RESULTS

A. Observations

1. Mortality - No rats died prior to scheduled terminal sacrifice.

2. Clinical signs - No treatment-related changes in the appearance or behavior of rats in any of the treatment groups compared to the controls were observed.

B. Body weight and body weight gain

As can be seen in table 1, the 1000 ppm group males and females had mean final body weights 8-9% lower (46.5 and 25.4 g, respectively) than the controls. For both sexes, mean body weights (adjusted for initial body weights) were significantly ($p \leq 0.05$ or $p \leq 0.01$) lower at weeks 2-14 compared to the controls. The 250 ppm group males and females had mean final body weights 5-6% lower (32.5 and 10.5 g, respectively) than the controls. Mean body weights were significantly ($p \leq 0.05$) lower for males at weeks 7-14 and for females at weeks 9 and 12-14 compared to the controls. The 75 ppm group males and females had mean body weights similar to the control mean body weights.

Table 1 Body Weight (g) and Weight Gain (g) for Selected Time Points[1]

	Male				Female			
WEEK	0	75ppm	250ppm	1000ppm	0	75ppm	250ppm	1000ppm
1	205.8	208.8	204.9	205.2	159.3	159.5	162.6	155.6
5	373.5	377.9	361.9 (3)	348.2** (7)	221.2	221.6	214.7 (3)	201.2** (9)
9	466.8	465.5	443.1* (5)	423.5** (9)	252.5	246.4	239.3* (5)	228.1** (10)
14	539.2	529.7	507.6* (6)	493.3** (9)	276.7	272.9	263.2* (5)	254.9** (8)
total gain	333.4	320.9 (4)	302.7 (9)	288.1 (14)	117.4	113.4(3)	100.6(14)	99.3(15)
1-5	167.7	169.1	157(6)	143(15)	61.8	62.1	52.1(16)	45.6(26)
5-9	93.3	98.6	81.2(13)	75.3(19)	31.3	24.8	24.6(21)	26.9(14)
9-14	72.4	64.2	64.5(11)	69.8(4)	24.2	26.5	24	26.8

1 Values in () are percent decrease compared to controls

* $p < 0.05$; ** $p < 0.01$ (statistics only done on body weight values)

C. Food consumption and compound intake

1. Food consumption - Mean daily food consumption (g/kg body weight/day) by rats in the 1000 ppm treatment groups was lower than food consumption by control rats throughout the study. Males consumed up to 6% less food than the controls throughout the study, and females consumed 7-20% less food than the controls throughout the study. The decreases were significant ($p \leq 0.05$ or $p \leq 0.01$) only for females at most weeks from weeks 2-10. Food consumption by the 250 and 75 ppm group rats was similar to food consumption by the respective control group throughout the study.

   Males in all treatment groups had feed efficiencies significantly lower than the controls at various weekly intervals during the study. The 1000 ppm group males had mean feed efficiency values 13-15% lower than the control values at weeks 1-4 and 5-8 ($p \leq 0.01$); the values were similar to control values at weeks 9-13. The 250 ppm group males had mean feed efficiency values 7-12% lower than the control values throughout the study; the decreases were significant ($p \leq 0.05$ or $p \leq 0.01$) at weeks 1-4 and 5-8. The 75 ppm group males had mean feed efficiency values 8-11% lower than the control values at weeks 5-8 ($p \leq 0.01$) and weeks 9-13 (not significant). Although females in all treatment groups had mean feed efficiency values up to 23% lower than the controls on occasion, the differences did not reach statistical significance. The 1000 ppm group females had mean feed efficiency values 23% lower than the controls at weeks 1-4; the values were similar to control values at weeks 5-13. The 250 ppm group females had mean efficiency values 14-17% lower than the control values at weeks 1-8; the values were similar to the controls at weeks 9-13. The 75 ppm group females had mean feed efficiency values similar to the control values except at weeks 5-8 when they were 19% lower than the control values.

2. Compound intake - Calculated compound consumption by male rats in the 75, 250, and 1000 ppm treatment groups averaged 5.6, 19.2, and 77.1 mg/kg/day, respectively. Calculated mean compound consumption by the corresponding female groups was 6.6, 21.8, and 84.4 mg/kg/day, respectively.

D. Functional Observational Battery

No treatment-related differences in FOB testing were observed in any of the treatment groups.

E. Motor Activity Measurements

No differences in motor activity measurements between the treated and control groups were considered treatment-related. Slight increases in mean motor activity levels in the 1000 ppm group males during weeks 5 and 9 and females during week 9 returned to control levels at week 14, indicating that the increases were not treatment-related.

F. Cholinesterase Activity

No treatment-related differences in brain, erythrocyte, and plasma cholinesterase activities or on neuropathy target esterase activity were observed in the 1000 ppm group rats compared to the control rats.

G. Sacrifice and Pathology

No treatment-related differences in absolute or relative brain weights were observed between rats in the treatment and control groups. No treatment-related gross postmortem differences were observed between rats in the treated and control groups. All abnormalities appeared to occur randomly and sporadically in all study groups. No treatment-related microscopic findings were observed in the nervous systems of rats in the 1000 ppm treatment groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that pirimicarb reduced growth, food consumption and/or food utilization in rats fed 250 or 1000 ppm in the diet for 90 days. No neurotoxic effects, quantitative or qualitative changes in FOB parameters or locomotor activity or treatment-related central nervous system changes were observed. The NOAEL for neurotoxicity was established at 1000 ppm for both sexes.

B. Reviewer's Discussion

We agree with the study author's conclusion that pirimicarb had no neurotoxicological or neuropathological effects on rats fed 75, 250 or 1000 ppm in the diet for 90 days. No clinical signs, changes in FOB or motor testing results suggested neurotoxic effects. Brain weight, length, and width of treated rats were unaffected. Brain, erythrocyte or plasma cholinesterase and neuropathy target esterase activities of the 1000 ppm group rats did not differ from corresponding control activities. Pirimicarb did exert toxic effects on body weights, food consumption and/or feed efficiency of rats treated at 250 or 1000 ppm.

The 1000 ppm treatment group males and females had mean body weights that were significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than the controls at most weekly intervals. Mean final body weights for both sexes were 8-9% lower than the control weights. Throughout the study, food consumption was reduced $\leq 6\%$ for males and 7-20% for females compared to corresponding control food consumption. The decreases were significant ($p \leq 0.05$ or $p \leq 0.01$) for females only at most weekly intervals. Mean feed efficiency values for males were 13-15% lower during the weeks 1-8, and for females were 23% lower during weeks

1-4 compared to the controls.

The 250 ppm treatment groups had mean body weights that were significantly ($p \leq 0.05$) lower for males at weeks 7-14, and for females at weeks 9 and 12-14 compared to the respective controls. Mean final body weights for both sexes were 5-6% lower than the control weights. Although food consumption was unaffected by treatment, both sexes had reduced feed efficiency values compared to the controls. Mean feed efficiency values were 7-12% lower for males throughout the study, and 14-17% lower for females during weeks 1-8 compared to control values.

The 75 ppm treatment groups did not appear to be affected by treatment. Although mean feed efficiency values were lower for males during weeks 5-13 (8-11%), and for females during weeks 5-8 (19%) compared to concurrent control values, mean body weights and food consumption values were unaffected by treatment.

In conclusion, no neurotoxicological or neuropathological effects were observed in any of the treatment groups. The toxicological LOAEL for this study is 250 ppm (19.2 mg/kg/day), based on decreased body weights and food consumption in both sexes. The toxicological NOAEL is 75 ppm (5.6 mg/kg/day). This conclusion is based on the lack of effects on body weights noted in this study, in addition to the lack of gross or histopathological findings noted in the subchronic oral toxicity study (MRID 44233103) reviewed in conjunction with this study.

IV. STUDY DEFICIENCIES

No positive control data were provided in the submission. However, acceptable positive control data from previous studies (MRIDs 43013301, 43013302, 43013303, 43013304, and 43013305) were available for comparison purposes. Therefore, this study is considered scientifically valid, and in conjunction with the available positive control data, can be used to meet guideline requirements.

SignOff Date:	9/1/99
DP Barcode:	D236012
HED DOC Number:	013708
Toxicology Branch:	RAB1

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DP Barcode:	D236012
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