EPA Reviewer: Laurence D. Chitlik, DABT, Date 9/11/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Marion Copley , Date 9/11/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE:21-Day Dermal Toxicity in the Rat; OPPTS 870.3200 (rodent), [§82-2]

DP BARCODE: D222462 SUBMISSION CODE:S499595
P.C. CODE: 106101 TOX. CHEM. NO.:359C

TEST MATERIAL (PURITY): Pirimicarb Technical 97.6% used as a paste in deionized water

SYNONYMS: Assigned CTL reference number Y00032/026

CITATION: D. Lees, Study Director
A.M. Leah, Author, 12/27/95.
Laboratory name: ZENECA Central Toxicology Lab
Alderly Park, Macclesfield, Cheshire, UK
Laboratory report number, CTL/P/4805.
MRID: 43896201. Unpublished

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY:

In a 21-Day dermal toxicity study (MRID [43896201]) Pirimicarb, (97.6 %a.i.) was administered dermally to 5 male and 5 female Alpk:APfSD (Wistar derived) rats per dose group as a paste in distilled water at dose levels of 0, 40, 200 and 1000 mg/kg/day. A total of 15 six-hour applications were made during the 21-day test period. Animals were fitted with plastic collars to prevent oral exposure.

Systemic toxicity was not observed up to and including 1,000 mg/kg/day. **The systemic LOEL is > 1,000 mg/kg/day and the systemic NOEL = 1,000 mg/kg/day.**

In addition, reductions in plasma and brain cholinesterase were observed in males and females in a dose related manner at all test levels. At the 1000 mg/kg dose level, brain cholinesterase was reduced 18.4% in males and 22.5% in females while at the 200 mg/kg/day dose level, reductions were 10.5% in males and 11.3% in females. Similarly, at the 1000 mg/kg dose level plasma

cholinesterase was reduced 19.6% in males and 39.7% in females while reductions were 17.6% in males and 23.4% in females at the 200 mg/kg dose level. At the low dose level (40 mg/kg) reductions were less than 5% for brain cholinesterase and less that 15% for plasma cholinesterase in both sexes. **Based upon these findings, the ChE LOEL in this study is considered 200 mg/kg/day and the ChE NOEL is the low dose level, 40 mg/kg/day.**

This 21-day dermal study is classified as acceptable and satisfies the guideline requirement for a 21-day dermal study (82-2) in rats. (See discussion and deficiency sections of this review)
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, were provided. A flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Reported to be Pirimicarb Technical
   Description: None provided
   Lot/Batch #: Reported to be Y00032/026 but then given # Y04517/015 and described as a paste in deionized water
   Purity: Reported to be 97.6% w/w, but a certificate of analysis not provided.
   Stability of compound: No data provided, however the report stated that "from information provided by the Sponsor, the test sample remained stable for the duration of the study."

2. Vehicle and/or positive control: deionized water

3. Test animals: Species: rat
   Strain: Alpk:APfSD
   Age and weight at study initiation: Age not specified. Males weighed 245-287 g and females weighted 197-224 g
   Source: The Specific Pathogen Free colony maintained at the Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderly Park

   Housing: individually in suspended stainless steel cages
   Diet: (Porton Combined Diet, Special Diet Services Ltd) ad libitum
   Water: tap water via an automatic watering system ad libitum
   Environmental conditions: Temperature: 21±2°C
   Humidity: 55±15%
   Air changes:25-30/hour
   Photoperiod:12 hours light
   Acclimation period: 12-13 days prior to study initiation

B. STUDY DESIGN:

1. In life dates - start: 23 August, 1995
end:14 September,1995

2. Animal assignment

Animals were assigned to each of 10 replicates (randomized blocks) each consisting of 4 cages, one per treatment group. The cages within each replicate were randomly allocated to a treatment group. Animals at the extreme of the weight range were discarded. Sexes were randomized separately. Sixteen to twenty-four hours before application of the test sample, the hair was removed with a pair of clippers from an area approximately 10cm x 5cm on the dorso-lumbar region. The amount of test material was calculated for each animal according to its weight at the time of dosing. The test material was made into a paste by adding a small amount of deionized water. One applied, the paste was covered a gauze patch which was kept in contact with the animal for approximately 6 hours using an occlusive dressing (plastic film) held in place by an adhesive bandage (2 pieces of pvc tape). Controls were similarly treated.

At the end of the 6-hour period, the dressings were cut and removed. The skin at the test site was cleansed using cotton soaked in warm water and then dried with clean tissue.

A total of 15 6-hour applications were made during this 21 day study. There were 3 two-day periods during which the animals were not dosed. Plastic collars were used to prevent oral exposure.

TABLE 1: STUDY DESIGN

Test Group	Color Code	Dose to Animal (mg/kg bw)	Male	Female
Control	blue	0	5	5
Low	green	40	5	5
Mid	yellow	200	5	5
High	red	1000	5	5

3. Test material preparation and analysis

There were no data in the report indicating storage or preparation of the test material other than the statement that deionized water was used. There were no actual analyses in the test report of the test material. Actual calculations for dosing of each animal were not included in the test report. Individual animal body weights were present in a appendix of the report.

Results - Homogeneity Analysis: no data available
Stability Analysis: no data available in the test report
Concentration Analysis: no data available in the test report

4. Statistics - *See attachment #1*

C. METHODS:

1. Observations:

Animals were inspected prior to dosing and after decontamination, and once daily on the days the animals were not dosed.

2. Body weight

Animals were weighed daily, before application of the test material "when appropriate".

3. Food consumption and compound intake

Food consumption for each animal was determined throughout the study and calculated on a daily basis.

4. Ophthalmoscopic examination

Eyes were not examined in this study.

5. Blood was collected at termination. Animals were bled by cardiac puncture. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*and distribution	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
x	activated partial(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium		Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)	x	Triglycerides
x	Cholinesterase (ChE)	x	Serum protein electrophores
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was NOT collected in this study.

The CHECKED (X) parameters were examined.

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

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for possible histological examination. All tissues were fixed in 10% neutral buffered formol saline, except for skin, testes and epididymides, which were fixed in Bouin's solution, and half the brain of each animal was placed on ice for measurement of cholinesterase activity. Note:Only kidney, liver, treated and untreated skin from all animals were routinely processed and embedded in paraffin wax. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*	x	Brain*Periph.
	Salivary glands*		Heart*		nerve*
	Esophagus*		Bone marrow*		Spinal cord (3
	Stomach*		Lymph nodes*		levels)^{T}
	Duodenum*		Spleen*		Pituitary*
	Jejunum*		Thymus*		Eyes (optic n.)^{T}
	Ileum*		UROGENITAL		GLANDULAR
	Cecum*				
	Colon*	xx	Kidneys*+		Adrenal gland*
	Rectum*		Urinary bladder*	x	Lacrimal gland^{T}
x	Liver*+	xx	Testes*+		Mammary gland^{T}
	Gall bladder*	x	Epididymides		Parathyroids*++
	Pancreas*		Prostate		Thyroids*++
	RESPIRATORY		Seminal vesicle		OTHER
	Trachea*		Ovaries		
	Lung*		Uterus*		Bone
	Nose				Skeletal muscle
	Pharynx			x	Skin
	Larynx				All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. **RESULTS**

A. Observations :

1. Toxicity - There were no signs of toxicity for skin irritation.

2. Mortality -None

B. Body weight and weight gain: There were no dose related effects on bodyweight.

C. Food consumption :

No compound related effects

D. Ophthalmoscopic examination - None performed

E. Blood work:

1. Hematology -Mean Cell Hemoglobin was slightly increased in a dose related manner in males reaching statistical significance at the 1000 mg/kg/day dose level. In addition, the Basophil Count was reduced in males of the high dose level. The eosinophil count was observed to decrease in both sexes, but differences were reported as statistically significant only for high dose females.(See attachment # 2). These hematological changes are considered equivocal by the RfD Committee.

2. Clinical Chemistry - Brain cholinesterase was reduced in a dose related manner at all test levels, but reached statistical significance only at the 1000 mg/kg dose level in both males (6.81 IU/g versus the control at 8.35 IU/g) and in females (8.17 versus 10.54 IU/g in controls). However, due to the small number of animals per group which would tend to limit statistical assessment, the trend should be more carefully considered in the selection of a NOEL for brain cholinesterase inhibition. At the 1000 mg/kg dose level, brain cholinesterase was reduced 18.4 % in males and 22.5% in females while at the 200 mg/kg/day dose level, reductions were 10.5 % in males and 11.3 % in females. (See attachment # 3)

In females, erythrocyte cholinesterase also decreased in a dose related manner at all dose levels although no statistical significance was reported. In males, a trend was not apparent. Plasma cholinesterase decreased in a dose related manner in both sexes and was reported as statistically significant at both the 200 and 1000 mg/kg/day dose levels. At the 1000 mg/kg dose level plasma cholinesterase was reduced 19.6% in males and 39.7% in females while reductions were 17.6% in males and 23.4% in females

at the 200 mg/kg dose level. At the low dose level (40 mg/kg) reductions were less than 5% for brain cholinesterase and less that 15% for plasma cholinesterase in both sexes.(See attachment #3)

Plasma Total Bilirubin was reduced in males at the 200 mg/kg/day dose level (not at the high dose level) and this finding is not considered associated with treatment. In addition, Plasma Alkaline Phosphatase decreased slightly in a dose related manner, but the finding reached significance only at the high dose level. Plasma GGT levels slightly increased with dose in males but no statistical significance was reported and no changes were apparent in females.

Plasma cholesterol was noted to increase only in high dose females at statistically significant levels ($p < 0.01$). (See attachment # 3)

F. Urinalysis - Not performed

G. Sacrifice and Pathology:

1. Organ weight - No compound related effects were apparent for kidney or liver in males or females. No effects were apparent in the testes. No other organs were assessed.

2. Gross pathology -No gross pathology was reported which appeared associated with administration of the test material. Data were tabulated only for the nasal cavity, skin and skull (Table 9).

3. Microscopic pathology - Histopathology findings were only presented for kidney, liver and skin in Table 10 of the test report. Only the high dose and control tissues were examined for these few tissues. No findings appeared associated with administration of the test material.

III. DISCUSSION

A. Although the pathological assessment in this study is generally inadequate, based up verbal communication with the primary reviewer of this chemical (G. Reddy, 2/26/96), the principle findings observed in oral studies are associated with hematology and clinical chemistry effects (primarily cholinesterase inhibition). Dr Reddy indicated that no target organs had been identified in other oral toxicity studies other than the toxicity observed in the bone marrow. Therefore, it will not apparently be necessary to require a

new study due to this deficiency.

In this 21-Day study, Mean Cell Hemoglobin slightly increased with dose in males reaching statistical significance at the 1000 mg/kg/day dose level (18.2 in controls versus 19.0 pg at the high dose level). Also in males, the Basophil Count was noted as reduced at the high dose level. The eosinophil count was reduced in males and females but only reached statistical significance in females at the 1000 mg/kg/day dose level. Thes

e hematological changes are considered equivocal by the RfD Committee.

The LOEL in this study is based upon reductions in plasma and brain cholinesterase observed in males and females at the 1000 and 200 mg/kg/day dose levels. Therefore the LOEL is 200 mg/kg/day and the NOEL is the low dose level, 40 mg/kg/day.

B. Study deficiencies

1. The batch number used in this study was not identified. In addition the certificate of analysis was not included as a reference and there are two CTL reference numbers used for identification of the test material. Therefore, there was some question as to the actual test material although stated to be 97.6% w/w Pirimicarb. On 2/26/96, Saundra O'brien of Zeneca AG Products was notified of this deficiency and agreed to provide clarification documents as to the test material used in this study.

On 2/27/96, additional data were received via fax from Saundra M. O'Bryan. These data included the certificate of Analysis dated 9/21/95 specifying that Y00032/026 is 97.6% w/w pirimicarb. These data indicated that the analysis was performed a week after the in-life portion of this 21 day study was completed (Attachment 4). On 2/29/96, other information was received from Ms O'bryan that defined Y04517/015 as deionized water (Attachment 5). Therefore, this question has been resolved.

2. Both the number of tissues taken for histopathological examination and the number of tissues actually receiving histopathological examination were inadequate.

3. The tabulation of gross findings was inadequate and only covered the nasal cavity, skin and the skull. However, individual data suggested that a complete necropsy was performed on each animal.

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