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DATA EVALUATION REPORT

STUDY TYPE: 90-day oral - Dog (82-1)

TOX. CHEM. NO.: 219AA

P.C. CODE: 059101

MRID NO.: 421728-01

TEST MATERIAL: Chlorpyrifos

SYNONYMS: Pyrinex

STUDY NUMBER: MBS 31/88999

SPONSOR: Makhteshim-Agan (America)

TESTING FACILITY: Huntingdon Research Centre, Ltd.
P.O. Box 2
Huntingdon, Cambridgeshire
PE18 6ES England

TITLE OF REPORT: Chlorpyrifos Oral Toxicity Study in Beagle Dogs (Repeated Daily Dosage for 13 Weeks)

AUTHOR: Michael H. Barker

REPORT ISSUED: 3 May 1989

CONCLUSIONS: Male and female pure-bred Beagle dogs were given the test article daily for 13 weeks at dosages of 0, 0.01, 0.22 or 5.0 mg/kg/day. The primary toxic effect was the inhibition of plasma, RBC and brain cholinesterase.

	MALE	FEMALE
NOEL	0.01 mg/kg/day (LDT)	0.01 mg/kg/day (LDT)
LOEL	0.22 mg/kg/day (MDT)	0.22 mg/kg/day (MDT)

CLASSIFICATION: core - Guideline

LOEL is based on significant inhibition (> 20% of control value) of both plasma and RBC cholinesterase.

This study does satisfy guideline requirements (82-1) for a 90-day feeding study in dogs.

A. MATERIALS:

1. Test compound: Chlorpyrifos, technical Description: Off-white waxy solid, Batch #: 489205, Purity: 95.8% Contaminants: list in CBI appendix

2. Test animals: Species: Dog Strain: Pure-bred beagles Age: 30 to 34 weeks Weight (kg): 9.6 - 13.2 (males), 9.1 - 12.2 (females) Source: Interfauna UK Ltd, Abbots Ripton Rd, Wyton, Huntingdon, UK.

B. STUDY DESIGN:

1. Animal assignment: Animals were assigned randomly to test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group	Dose in Diet (mg/kg/day)	Main Study (13 weeks)	
		Male	Female
Control (CON)	0	4	4
Low (LDT)	0.01	4	4
Mid (MDT)	0.22	4	4
High (HDT)	5.0	4	4

2. Dosage: Test article was mixed with lactose (Thorton & Ross, Linthwaite Laboratory, Huddersfield, UK, Lot No. JO18, Exp 11 Feb 90) to yield a final concentration of 1 %. Samples of the mixture were analyzed and found to be homogeneous (1.18 % coefficient of variation) and within 98.3 and 104 % of the nominal concentration. Appropriate amount of test article-lactose mixture was weighed and placed in gelatin capsules (Parke-Davis prefit capsules, size 000). Dogs in the control group received 500 mg/kg/day of lactose. Dogs received one capsule/day for the duration of the study.

3. Animals received 400 grams of standard dry diet (Diet A, Specialty Diets Services) each day and water ad libitum. During the dosing period, it became necessary to moisten the diet of some dogs with an equal amount of hot water, in an attempt to stimulate appetite. One dog was given canned meat during week 12 and a mixture of dry diet, canned meat and hot water during week 13.

4. Statistics: The data were first analyzed using the Bartlett's test and if found to be homogeneous, a one-way analysis of variance (ANOVA) was performed. Heterogeneous data were reevaluated following a logarithmic

transformation. Data still possessing significant heterogeneity after being transformed were analyzed using the Kruskal-Wallis analysis of ranks followed by the Shirley's test. Post hoc analysis of homogeneous data consisted of Student's t-test or Williams' test for dose-related effects.

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

6. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effefcts to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected regularly throughout the day for signs of toxicity, moribundity and mortality.

a. Toxicity: There were no clinical observations attributable to the administration of the test article. Occasional liquid feces and vomiting were observed in both control and treated animals.

b. Mortality (survival): All animals survived until the scheduled sacrifice.

2. Body weight: Animals were weighed weekly during the 13 week treatment period.

Results: There were no significant dose-related changes in the body weights of the male and female dogs. Animals in the 5 mg/kg/day group showed a small, but insignificant, weight loss over the course of the study.

3. Food consumption and compound intake: Food consumption was determined weekly and mean daily consumption was calculated.

a. Food consumption results: Daily food consumption (g/animal/day) for both male and female dogs was not altered by treatment.

b. Food efficiency results: Not determined.

4. Ophthalmological examinations: Examinations were performed on all animals at the termination of the study. No treatment-related eye lesions were observed.

5. Hematology and Clinical Chemistry: Clinical chemistry analyses were performed during the pre-dosing period, after weeks 1 and 6 of dosing period and at the termination of

the study. The checked (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Prothrombin time
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc.(MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)
X	Activated Partial Thromboplastin Time (APTT)	X	Bone marrow

Results: No findings were considered to be treatment-related. Although statistically significant differences were found, no meaningful biological changes were evident.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Enzymes	X	Total Bilirubin
X	Alkaline phosphatase		Triglycerides
X	Cholinesterase	X	Total Protein
	Creatinine phosphokinase		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT/ALT)		
X	Serum aspartate aminotransferase (SGOT/AST)		

Results: With the exception of the cholinesterase levels, none of the other biochemical parameters was considered to be biologically significant. Both plasma and RBC cholinesterase activities showed highly significant, dose-related changes during the course of the study (Table 2, below). Brain cholinesterase activity was also significantly inhibited in the 5.0 mg/kg/day group (Table 3).

6. Urinalysis: Urinalysis was performed during the pre-dosing period, after 6 weeks of exposure and at the termination of the study. The checked (X) parameters were examined.

X	Volume	X	Glucose
X	Specific gravity	X	Ketone Bodies
X	Protein	X	Bile Pigments
X	Appearance	X	Urobilirubin
X	Sediment	X	Total Bilirubin
X	pH	X	Blood

Results: No treatment-related effects were noted. A transient increase in urine protein was seen at week 6 in the male animals in the 0.22 and 5.0 mg/kg/day dose groups.

7. Sacrifice and Pathology: Detailed pathological examination was performed on male and female dogs in the control and treatment groups. The checked (X) tissues were collected for histological examination; the checked (XX) organs were also weighed.

Digestive system	Cardiovas./Hematol	Neurologic
X Tongue	X Aorta	XX Brain
X Salivary glands	XX Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord (3x)
X Stomach	X Lymph nodes	XX Pituitary
X Duodenum	XX Spleen	X Eyes
X Jejunum	XX Thymus	Glandular
X Ileum	Urogenital	XX Adrenals
X Cecum	XX Kidneys	Lacrimal gland
X Colon	X Urinary bladder	X Mammary gland
X Rectum	X Testes	X Parathyroids
XX Liver	Epididymides	XX Thyroids
X Gall bladder	X Prostate	Other
X Pancreas	Seminal vesicle	X Bone
Respiratory	X Ovaries	X Skeletal muscle
X Trachea	X Uterus	X Skin
XX Lungs	X Vagina	X Gross lesions
Nasal Passages	Cervix	

a. Organ Weights: The organ weights of the treated animals were not significantly different from the control values.

b. Gross Pathology: Minor changes, not related to treatment, were observed in some of the control and treated animals. In the 5.0 mg/kg/day group, one female showed thickening of the wall of the duodenum and jejunum, while one male showed multiple red raised foci on the mucosal surface throughout the duodenum and duodenal mucosal thickening. These effects may be treatment-related.

c. Microscopic Pathology

1) Non-neoplastic: The animals which showed gross pathological changes, as described above, also showed possible treatment-related histopathological changes. The male showed thickened muscular coat of the duodenum, while the female showed thickened muscular coat of the duodenum and an area of papillomatous hyperplasia (pyloric).

Table 2: Plasma and RBC cholinesterase (ChE) activity (μmol/ml/min) for week number and hours after dosing. Cholinesterase activity was measured 3 and 24 hours after administration of the test article.

	Dose	Pre Dose	Week 1		Week 6		Week 12	
Male			3 hr	24 hr	3 hr	24 hr	3 hr	24 hr
Plasma ChE	0	1.50	1.45	1.56	1.44	1.43	1.52	1.38
	0.01	1.60	1.47	1.54	1.38	1.27	1.30	1.18
	0.22	1.65	0.89**	1.04**	0.64**	0.79**	0.56**	0.80**
	5.0	1.45	0.39**	0.49**	0.22**	0.37**	0.33**	0.41**
RBC ChE	0	2.29	2.31	2.22	2.07	2.18	2.21	2.13
	0.01	2.30	2.22	2.18	1.94	2.16	2.11	1.91
	0.22	2.02	1.93	2.00	1.37**	1.47**	1.41**	1.15**
	5.0	2.03	1.43**	1.35**	0.32**	0.41**	0.37**	0.31**
Female			3 hr	24 hr	3 hr	24 hr	3 hr	24 hr
Plasma ChE	0	1.59	1.65	1.70	1.68	1.48	1.62	1.57
	0.01	1.57	1.61	1.56	1.27*	1.19*	1.23	1.20
	0.22	1.70	0.79**	0.98**	0.59**	0.71**	0.54**	0.75**
	5.0	1.69	0.43**	0.61**	0.22**	0.40**	0.26**	0.40**
RBC ChE	0	2.17	2.21	2.21	2.04	2.13	2.30	1.99
	0.01	1.89	1.94	2.00	1.74	1.94	1.91	1.63
	0.22	2.00	1.90	1.88	1.40**	1.62*	1.43**	1.27**
	5.0	2.03	1.56**	1.54*	0.28**	0.31**	0.44**	0.32**

* $p \leq 0.05$, ** $p \leq 0.01$

Table 3: Brain Cholinesterase Activity

Dose	Brain Cholinesterase (μmol/g/min)	
	Male	Female
0	3.64	3.31
0.01	3.37	3.49
0.22	3.38	3.00
5.00	1.95**	1.78**

* $p \leq 0.05$, ** $p \leq 0.01$

2) Neoplastic: Not noted

D. DISCUSSION: Male and female dogs were treated orally with the test article (0, 0.01, 0.22 and 5.0 mg/kg/day) for 13 weeks. The test article significantly inhibited plasma, RBC and brain cholinesterase.

	MALE	FEMALE
NOEL	0.01 mg/kg/day (LDT)	0.01 mg/kg/day (LDT)
LOEL	0.22 mg/kg/day (MDT)	0.22 mg/kg/day (MDT)

LOEL based on inhibition of plasma and RBC cholinesterase.

Classification: core - Guideline

This study does satisfy guideline requirements (82-1) for a 90-day feeding study in dogs.