UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 27 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Chlorpyrifos: Review of submitted data to support registration

EPA IDENTIFICATION NUMBERS: Caswell No.: 219AA
P.C. Code: 059101
D.P. Barcode: D184203

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 23 apr 93.
Toxicology Branch II, Section IV
Health Effects Division

TO: Dennis Edwards
Product Manager (19)
Registration Division (H7505C)

THRU: Elizabeth Doyle, Ph.D. E. A. Doyle 4/22/93
Toxicology Branch II, Head Section IV
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. M van Gemert 4/27/93
Chief, Toxicology Branch II

Registrant: Makhteshim-Agan (America)

Chemical: Chlorpyrifos (Pyrinex)

Action Requested: Review toxicology studies on chlorpyrifos (pyrinex) submitted by Makhteshim-Agan (America) to support registration.

1. The following study was reviewed:

Pyrinex Technical Oncogenicity Study in the Mouse (MRID NO.: 425342-01)

Results: This study evaluated the oncogenic potential of test compound, at dietary concentrations of 0, 5.0, 50 or 250 ppm (equivalent to approximately 0, 0.89, 8.84, or 45.2 mg/kg/day for males and 0, 0.938, 9.79, or 48.1 mg/kg/day for females, respectively) when administered to CD-1 mice for 78 weeks.

Systemic toxicity was observed in high-dose animals and included decreased body weight and feed consumption in males, lower mean water consumption in females, increased incidence of gross clinical findings (ocular opacity, hair loss on head and around eyes) and non-neoplastic lesions (keratitis, hepatocytic fatty vacuolation) in high-dose males and females. Neoplastic lesions were observed in both sexes, but were not considered to be treatment-related. Plasma cholinesterase activities were significantly reduced at all treatment levels; brain activities were significantly decreased only in the high-dose animals. Results of the study showed that the test compound does not have oncogenic potential.

	NOEL	LOEL
Systemic toxicity	50 ppm (MDT)	250 ppm (HDT)

The systemic LOEL is based on decreased body weights in males, increased incidence of non-neoplastic lesions in males and females.

Classification: core - Guideline

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 2 Mar 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 3/2/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Oncogenicity study in mice (83-2)
CASWELL NO.:	219AA
P.C. CODE:	059101
MRID NO.:	425342-01
TEST MATERIAL:	Chlorpyrifos
SYNONYMS:	Pyrinex, O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
STUDY NUMBER:	MAK/106/PYR
SPONSOR:	Makhteshim-Agan (America)
TESTING FACILITY:	Life Science Research Israel, Ltd. Ness Ziona 70451 Israel
TITLE OF REPORT:	Pyrinex Technical Oncogenicity Study in the Mouse
AUTHORS:	E. Gur
REPORT ISSUED:	15 October 1992

CONCLUSIONS: This study evaluated the oncogenic potential of test compound, at dietary concentrations of 0, 5.0, 50 or 250 ppm (equivalent to approximately 0, 0.89, 8.84, or 45.2 mg/kg/day for males and 0, 0.938, 9.79, or 48.1 mg/kg/day for females, respectively) when administered to CD-1 mice for 78 weeks. Systemic toxicity was observed in high-dose animals and included decreased body weight and feed consumption in males, lower mean water consumption in females, increased incidence of gross clinical findings (ocular opacity, hair loss on head and around eyes) and non-neoplastic lesions (keratitis, hepatocytic fatty vacuolation) in high-dose males and females. Neoplastic lesions were observed in both sexes, but were not considered to be treatment-related. Plasma cholinesterase activities were significantly reduced at all treatment levels; brain activities were significantly decreased only in the high-dose animals. Results of the study showed that the test compound does not have oncogenic potential.

	NOEL	LOEL
Systemic toxicity	50 ppm (MDT)	250 ppm (HDT)

The systemic LOEL is based on decreased body weights in males, increased incidence of non-neoplastic lesions in males and females.

Classification: core - Guideline

This study satisfies guideline requirements (83-2) for an oncogenicity study in mice.

I. MATERIALS and METHODS

A. Test compound: Chlorpyrifos, technical Description: off-white solid Batch #: 289318 Purity: 95.9% Contaminants: Not given

B. Test animals: Species: Mouse Strain: CD-1 Age: 3 weeks Weight (g): 11.0 - 17.0 (males), 9.7 - 17.0 (females) Source: Charles River Breeding Laboratories, UK

C. Diet Preparation: A weighed amount of test compound was melted (water bath, 50-60 °C) and dissolved in corn oil. The solution was mixed with basal diet (Altromin 1321N, Altromin International, Ltd, Lage, Germany) to form a premix. The premix was mixed with appropriate amounts of basal diet to form the desired final concentration of test compound in each of the test diets. The control diet was prepared by mixing corn oil only with basal diet at a concentration equal to that used to prepare the high dose diet. All diets were prepared weekly.

Before the study was started, trial test diets were prepared and analyzed for homogeneity, concentration and stability. Diets were mixed to homogeneity (relative standard deviations of 8.2, 6.0 and 2.7% for 5, 50 and 250 ppm diets, respectively). The target doses for the trial diets ranged from 3.8 to 5.4 ppm, 33.5 to 51.6 ppm and 200 to 254 ppm for 5.0, 50 and 250 ppm diets, respectively. The amount of test compound in the diets was not corrected for percent purity.

D. Study Design

1. Animal assignment: The assignment of animals to study groups is summarized in Table 1. Animals were housed four/cage, sexes separate.

Table 1: Animal Assignment to Study Groups

Study Group	Dose in Diet (ppm)[b]	Main Study		ChE Assay[a]	
		Male	Female	Male	Female
Control (CON)[c]	0	59	59	5	5
Low (LDT)	5	59	59	5	5
Mid (MDT)	50	59	59	5	5
High (HDT)	250	59	59	5	5

a. During Week 42 of the study 5 animals/group/sex were sacrificed for determination of plasma, RBC and brain cholinesterase activities.
b. Not corrected for percent purity of test compound
c. Control diets contained corn oil at a concentration equal to that used for preparation of the high-dose diet.

E. Statistics: Data were initially analyzed for homogeneity of variances using Bartlett's test. Homogeneous data were analyzed using analysis of variance (ANOVA); significant ANOVA results were further analyzed using Dunnett's test. Heterogeneous data were analyzed using Kruskal-Wallis' nonparametric ANOVA. Significant results were further analyzed using either Dunn's test or the Wilcoxon test. The statistical methods used to evaluate the pathology incidence data were presented in detail in the Statistician's Report in the study using the procedures recommended by Peto et al. (International Agency for Research Against Cancer. Monographs: Long-term and short-term screening assays for carcinogens: A critical appraisal. WHO, Geneva, 1980, Supplement 2, pp 311-426).

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

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

II. RESULTS

A. Observations: Animals were inspected daily for signs of toxicity, moribundity and mortality. Detailed examinations were preformed at least weekly.

1. Toxicity: Significant, treatment-related increases in the incidence of gross clinical observations are summarized in Table 2. Excessive lachrymation and ocular opacity were noted in males and females (Table 2). A higher incidence of hair loss on the head and around the eyes was noted in males only.

Table 2: Gross clinical observations (Data summarized from Table 3 of the study)

Observation	Sex	CON	LDT	MDT	HDT
Excessive lachrymation	Male	2/64	4/64	7/64	16/64*
	Female	0/64	2/64	0/64	6/64*
Ocular opacity	Male	0/64	3/64	8/64*	10/64*
	Female	3/64	1/64	3/64	10/64*
Hair loss on head	Male	0/64	2/64	4/64	9/64*
Hair loss around eyes	Male	4/64	6/64	10/64	18/64*

* $p \leq 0.05$, calculated by reviewer using chi-squared analysis.

2. Mortality (survival): Cumulative deaths and humane and moribund sacrifices occurring through Week 78 are summarized in Table 3. No treatment-related effects were noted, however, the percent survival of the high-dose males was significantly higher than the control value.

Table 3: Animal mortality (% survival) through Week 78 (Data summarized from Tables 1 and 2 of the study)

Sex	CON	LDT	MDT	HDT
Male	33 (48%)	38 (41%)	27 (58%)	15 (77%*)
Female	16 (75%)	11 (83%)	17 (73%)	18 (72%)

* $p \leq 0.05$

C. Body weight: Animals were weighed at the start of the study, routinely for the first 13 weeks, monthly, thereafter, and at terminal sacrifice. Body weight data are presented in Appendix 1 of this review. Consistent, significant decreases in mean body weights were observed throughout the study for the high-dose males. Occasional, significant increases were noted in the low-dose males. Mean terminal body weight of the high-dose males (38.22 g) was significantly ($p \leq 0.01$) lower than that of the controls (41.49 g). The only significant decreases in the mean female body weight occurred during the first three weeks of the study, suggestive of food palatability effect rather than a treatment-related effect. Body weight gain data were not presented in the study.

D. Water and food consumption, food conversion ratio, and compound intake: Food and water consumption were calculated by dividing the total amount consumed/cage by weekly cage population. Food and water consumption were determined weekly through Week 13 and monthly, thereafter. Food conversion ratios (body weight change/food consumption) were calculated only through Week 13 of the study.

1. Water consumption: Significant changes in water consumption are summarized in Appendix 2. While the high-dose females showed consistent decreases in water consumption throughout the study, the males showed only sporadic decreases.

2. Food consumption: Food consumption by high-dose males was lower than control values throughout most of the study and was significantly lower during Weeks 1, 2, 3, 21, 25, 37, 65, and 73 (Appendix 3). Treatment did not alter food consumption by any of the females.

3. Food conversion ratio: No significant differences were noted in the food conversion ratios of any of the study groups.

4. Compound intake: The mean compound intake data are summarized in Table 4, below.

Table 4: Compound Intake (mg/kg body weight/day) (Calculated from data presented in Table 7 of the study)

Study Group	Compound Intake	
	Male	Female
LDT	0.890	0.938
MDT	8.84	9.79
HDT	45.2	48.1

E. Cholinesterase activity: Plasma, erythrocyte (RBC) and brain cholinesterase activities (5 animals/sex/group) were measured on Weeks 42 and 78. Significant results are presented in Table 5, below.

NOTE: Different assay methods were used to evaluate RBC cholinesterase activity at Week 42 and Week 78. For Week 42, RBC cholinesterase activity was measured on unwashed cell, whereas for Week 78 the RBC's were washed three times before lysis. Week 42 RBC cholinesterase activities are markedly higher than activities at Week 78 (presumably from plasma contamination of the RBC's). Since no explanation was given to justify the change in methods and no parallel comparison of the two methods was performed, the validity of the RBC cholinesterase activity is questioned by the reviewer.

Table 5: Cholinesterase Activity (Data from Tables 10 and 11 of the study)

Cholinesterase	Sex	Week	CON	LDT	MDT	HDT
Brain (U/g tissue)	Male	42	7.9	7.3	4.5	1.6**
		78	11.4	8.3	6.1	1.6**
	Female	42	9.6	8.3	5.2*	1.4***
		78	7.6	8.6	6.9	1.2***
Plasma (U/l)	Male	42	4918	2408***	226***	85***
		78	6343	3234*	295**	149***
	Female	42	9174	5037***	256***	85***
		78	9519	4784***	345***	158***

Significant difference compared to vehicle control: * $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$

F. Hematology: Differential white cells counts were performed on control and high-dose animals after 53 and 79 weeks of treatment. No significant differences were noted in eosinophil, lymphocyte, monocyte, neutrophil or normocyte counts.

G. Sacrifice and Pathology: Detailed gross pathological examinations were performed on animals sacrificed in moribund condition, dying during the study, or surviving to terminal sacrifice. All of the tissues listed below were fixed; selected tissues from control and high-dose animals were examined histologically (X). Histological examination was also performed on the eyes, kidneys, livers, lungs and thyroids of the low- and mid-dose animals. Tissues marked XX were also weighed before histological examination.

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

1. Organ Weights: Absolute and relative organ weights were measured at terminal sacrifice; no significant, treatment-related effects were noted.

2. Gross Pathology: The incidence of gross pathological observations and palpable masses, subsequently confirmed histologically as neoplasms, did not show any treatment-related effects and were considered to be spontaneous in nature.

3. Microscopic Pathology

a) Non-neoplastic lesions: The incidence of significant, non-neoplastic lesions is summarized in Table 7, below. The eyes appeared to be a primary target organ, with increased incidence of keratitis in high-dose males and females.

Table 6: Non-neoplastic lesions (Data summarized from Statistics Table4)

Lesion	CON	LDT	MDT	HDT
---------- MALES ----------				
Eyes:				
Keratitis	1/54	0/54	4/57	7/57*
Skin:				
Ulcerative dermatitis on head	1/59	3/37	6/38	11/59*
Liver:				
Hepatocytic fatty vacuolation	2/59	3/59	3/59	14/59***
Histiocytic proliferation	5/59	0/59	1/59	7/59*
---------- FEMALES ----------				
Eyes:				
Keratitis	0/57	0/58	0/57	3/58
Salivary glands:				
Lymphocytic infiltration	9/58	1/6	1/12	21/59**
Lungs:				
Accumulation of alveolar macrophages	3/59	2/59	3/59	14/59***
---------- COMBINED (MALE + FEMALE) ----------				
Eyes				
Keratitis	1/111	0/112	4/114	10/115***
Retinal atrophy	0/111	0/112	2/114	3/115*
Pano- & endophthalmitis	0/111	1/112	1/114	5/115***
Liver:				
Hepatocytic fatty vacuolation	4/118	4/118	5/118	14/118*
Pericholangitis	24/118	24/118	16/118	36/118*
Salivary glands:				
Lymphocytic infiltration	18/117	3/38	3/34	34/118*
Lungs:				
Accumulation of alveolar macrophages	8/118	6/118	7/118	19/118***
Peribronchial lymphoid hyperplasia	6/118	3/118	4/118	10/118*

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$: Significant differences from control

High-dose males also showed increased incidence of ulcerative dermatitis on the head, and fatty vacuolation and histiocytic proliferation of theliver. High-dose females had an increased incidence of alveolar macrophage infiltration, uterine endometriosis, and lymphocytic infiltration of the salivary glands. When lesions were combined by sex, significant increases in incidence of retinal atrophy, pano- and endophthalmitis, peribronchial lymphoid hyperplasia in the lung, and hepatic pericholangitis.

NOTE: The study indicates (page 35) that other lesions were noted in the control and treated animals, however, the changes were regarded as incidental and of no relation to treatment. To support this claim, the Registrant needs to supply historical incidence data for both neoplastic and non-neoplastic lesions in the CD-1 mouse.

b) Neoplastic lesions: Neoplastic lesions were noted in both males and females. The study indicated that the lesions were not considered treatment-related, because the frequency of lesions was low and within the published historical ranges for CD-1 mice.

III. **DISCUSSION**

This study evaluated the oncogenic potential of test compound, administered for 78 days to CD-1 mice at dietary concentrations of 0, 5.0, 50 or 250 ppm (equivalent to approximately 0, 0.89, 8.84, or 45.2 mg/kg/day for males and 0, 0.938, 9.79, or 48.1 mg/kg/day for females, respectively).

The body weights were significantly decreased in high-dose males throughout most of the study; terminal mean body weight of these animals was also significantly lower than controls. High-dose males also showed decreased food consumption throughout the study; food efficiency, however, was not altered by treatment. The changes in females mean body weights were sporadic in nature and not considered to be treatment-related. High-dose females did, however, show significantly lower mean water consumption during most of the study.

Because the assay method for RBC cholinesterase was changed during the study, without adequate explanation or justification, these data could not be reviewed. The RBC cholinesterase activity after 42 weeks of treatment were markedly higher than after 78 weeks. Since the RBC's assayed at Week 42 were unwashed, a significant amount of the activity present might be due to plasma cholinesterase. The registrant needs to verify the methodology for determination of RBC cholinesterase activity and

establish what effect washing has on activity.

There were significant increases in the incidence of non-neoplastic lesions in high-dose males and females. The increased incidence of ocular opacity, noted in mid- and high-dose males and females, was, in most cases, diagnosed as keratitis upon microscopic evaluation. Other significant eye lesions included retinal atrophy, pano- and endophthalmitis. In conjunction with the eye lesions, there was an increased incidence of skin ulceration on the head, particularly around the eyes. Increases in treatment-related lesions were also observed in the liver (hepatocytic fatty vacuolation, histiocytic proliferation and pericholangitis), lung (accumulation of alveolar macrophages and peribronchial lymphoid hyperplasia), salivary glands (lymphocytic infiltration) and uterus (endometriosis). Although the study states that treatment-related non-neoplastic lesions were limited to the eyes (keratitis) and liver (hepatocytic fatty vacuolation), no historical data were provided to verify the claim that other significant, non-neoplastic lesions were typical for CD-1 mice.

Although neoplastic lesions were noted in both males and females, they were not considered treatment-related. Results of the study showed that the test compound does not have oncogenic potential.

	NOEL	LOEL
Systemic	50 ppm (MDT)	250 ppm (HDT)

The systemic LOEL is based on decreased body weights in males, increased incidence of non-neoplastic lesions in males and females.

Classification: core - Guideline

This study satisfies guideline requirements (83-2) for an oncogenicity study in mice.

CHLORPYRIFOS

Page_____ is not included in this copy.

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