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

APR 30 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Chlorpyrifos: Review subchronic toxicity study submitted in response to data call-in notice

EPA IDENTIFICATION NUMBERS: Caswell No.: 219AA
P.C. Code: 059101
D.P. Barcode: D184012

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 27 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 4/27/93
Toxicology Branch II, Head Section IV
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia Gemert 4/28/93
Chief, Toxicology Branch II

Registrant: Makhteshim-Agan (America)

Chemical: Chlorpyrifos (Pyrinex)

Action Requested: Review subchronic toxicology study on chlorpyrifos (pyrinex) submitted by Makhteshim-Agan (America) in response to data call-in notice.

1. The following study was reviewed:

Pyrinex Technical Toxicity in Dietary Administration to Mice for 13 Weeks (MRID No.: 425258-01)

RESULTS: This study evaluated the subchronic toxicity of test compound at dietary concentrations of 0, 5, 50, 200, 400 or 800 ppm (≈ 0, 0.8, 8.1, 33.5, 67.2 and 141 mg/kg/day (males); 0, 1.0, 10.9, 43.9, 73.2, and 153 mg/kg/day (females) respectively) when administered to mice for 13

(1)

weeks. Because of food spoilage during the first four weeks of the study, the following parameters were considered to be invalid and therefore could not be evaluated: body weight data for the entire study and food consumption data for first four weeks of the study. Further, because of pooling of paired blood samples (without attention to aliquot volumes), plasma and erythrocyte cholinesterase activities could not be evaluated. The following treatment-related effects were noted at terminal sacrifice: significantly lower brain cholinesterase in both males and females at 50 ppm and higher doses, increased relative liver weights of females in the 200 ppm and higher dose groups, and increased incidence of non-neoplastic lesions (lipogenic pigmentation of the adrenal cortex and keratitis) in high-dose females.

2. Conclusions: Because of the problems associated with the study, the reviewer felt that there was inadequate data to assign a NOEL and LOEL.

Classification: core - Supplementary (Can not be upgraded)

This study does not satisfy guideline requirements (82-1) for a subchronic oral toxicity study in mice.

Reviewed by: Robert F. Fricke, Ph.D. Robert F Fricke 27 Apr 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E A Doyle 4/27
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

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

P.C. CODE: 059101 **CASWELL NO.:** 219AA

MRID NO.: 425258-01

TEST MATERIAL: Chlorpyrifos, Pyrinex

SYNONYMS: O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate

STUDY NUMBER: MAK/105/PYR

SPONSOR: Makhteshim-Agan (America)

TESTING FACILITY: Life Science Research Israel, Ltd.
Ness Ziona 70451 Israel

TITLE OF REPORT: Pyrinex Technical Toxicity in Dietary Administration to Mice for 13 Weeks

AUTHOR: S. Crown

REPORT ISSUED: 16 October 1992

CONCLUSIONS: This study evaluated the subchronic toxicity of test compound at dietary concentrations of 0, 5, 50, 200, 400 or 800 ppm (≈ 0, 0.8, 8.1, 33.5, 67.2 and 141 mg/kg/day (males); 0, 1.0, 10.9, 43.9, 73.2, and 153 mg/kg/day (females) respectively) when administered to mice for 13 weeks. Because of food spoilage during the first four weeks of the study, the following parameters were considered to be invalid and therefore could not be evaluated: body weight data for the entire study and food consumption data for first four weeks of the study. Further, because of pooling of paired blood samples (without attention to aliquot volumes), plasma and erythrocyte cholinesterase activities could not be evaluated. The following treatment-related effects were noted at terminal sacrifice: significantly lower brain cholinesterase in both males and females at 50 ppm and higher doses, increased relative liver weights of females in the 200 ppm and higher dose groups, and increased incidence of non-neoplastic lesions (lipogenic pigmentation of the adrenal cortex and keratitis) in high-dose females. Because of the problems associated with the study, the reviewer felt that there was inadequate data to assign a NOEL and LOEL.

Classification: core - Supplementary (Can not be upgraded)

This study does not satisfy guideline requirements (82-1) for a subchronic oral toxicity study in mice.

3

I. MATERIALS and METHODS

A. Test compound: Chlorpyrifos, technical Description: off-white granular crystals Batch #: 589009 Purity: see note Contaminants: Not given

NOTE: The study indicates that the purity of the test material is either 96.1% (container label) or 93.5% (physicochemical data provided by Sponsor).

B. Test animals: Species: Mouse Strain: CD-1 Age: Not given Weight (g): 14 - 20 (males), 12 - 18 (females) Source: Charles River Breeding Laboratories (UK), Ltd.

C. Diet Preparation: A weighed amount of test compound was melted (water bath, 50°C) and dissolved in corn oil preheated to the same temperature. The solution was mixed with basal diet (Altromin 1321N, Altromin Spezial-futterwerke GmbH, Lage, Germany) to form a premix. The premix was mixed with appropriate amounts of basal diet to form the 800 ppm test diet. The 5, 50, 200, and 400 ppm diets were prepared by serial dilution of the 800 ppm diet with basal diet. The amount of test material added was not adjusted for the purity. All diets were prepared weekly. Test diets were prepared and analyzed for homogeneity and stability before the study was initiated. The test diets were analyzed during Weeks 2, 4, 8 and 12 of the study.

No tables were included in the study which summarized the homogeneity, stability and concentration data. Although analytical reports (Addendum 4) are included with the study, it is not readily apparent to the reviewer which reports correspond to the evaluation of the stability and concentration. Further, it is not apparent what the test diet samples corresponded to, i.e. whether they were taken from the top, middle, or bottom. Cursory review of the analytical reports indicates that the target doses were never achieved and that there was no attempt to adjust the quantity of test material based on percent purity. The homogeneity data showed wide variations between the different test diets (Table 1).

Table 1: Homogeneity of Test Diets

Dose (ppm)			Relative
Target	Achieved	Range	S.D. (%)
5	4.0	3.4 - 4.9	14
50	42.9	42.9 - 46.3	2.8
200	192	172 - 266	19.2
400	363	324 - 435	11.2
800	698	665 - 740	4.6

D. Study Design: The assignment of animals to study groups is summarized in Table 2. Within each study group, animals were housed four per cage. Animals were exposed to test diets for at least 13 weeks; surviving animals were sacrificed during weeks 14 and 15.

Table 2: Animal Assignment to Study Groups

Test Group		Dose in Diet (ppm)	Main Study (13 weeks) Male	Female
Control	(CON)	0	12	12
Low	(LDT)	5	12	12
Middle 1	(MDT1)	50	12	12
Middle 2	(MDT2)	200	12	12
Middle 3	(MDT3)	400	12	12
High	(HDT)	800	12	12

E. Statistics: Student's t-test, using pooled within-group error variance, was used to evaluate intergroup differences of measured parameters.

NOTE: The use of Student's t-test to compare three or more groups is not valid. Bartlett's test for homogeneity, analysis variance and either Duncan's multiple range test or Dunnett's test would be more appropriate.

Calculations of a standard deviation when n = 2 is meaningless (Tables 4, 5, and 7). Data should be expressed as one-half of the range.

In Appendix 8 of the study, the brain weight of animal number 62 was not included in the statistical evaluation because it was considered an "outlying value". The study does not indicate the criteria or methods for determining the presence of outlying values.

There also seems to be a problem with rounding off data. For example, the individual animal data for body weights (Appendix 2) are expressed in three significant digits, while the summarized data (Table 3) show four significant digits. The same problem exists with data presented in Tables 4, 5, and 8.

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.

5

II. **RESULTS**

A. Observations: Animals were inspected twice daily (once daily during weekends and holidays) for signs of toxicity, moribundity and mortality. Detailed examinations were preformed at least weekly.

1. Toxicity: Gross clinical observations performed during the study are summarized in Table 3. Significant findings included higher incidence of urogenital staining in males at the two highest doses and ocular opacity in high dose females.

2. Mortality (survival): Treatment-related deaths occurred in 4 males and 2 females in the 800 ppm group and 2 females in the 400 ppm group. Other deaths were noted, but were not related to treatment.

3. Ophthalmological Examinations: Not performed.

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

Observation	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
Urogenital staining	M	1/12	0/12	0/12	1/12	9/12**	11/12***
Ocular opacity	F	0/12	0/12	0/12	0/12	1/12	7/12*

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

B. Body weight: Animals were weighed at the start of the study and weekly until terminal sacrifice.

Results: Interpretation of body weight data is not possible because of the change in the feeding protocol after Week 4 of the study. Although there is an apparent decrease in body weights for males and females in the two highest dose groups, this change is likely due to decreased food consumption due to food spoilage (see discussion below) rather than a treatment-related effect.

C. Food consumption, food conversion ratio, compound intake and water consumption: Food consumption was determined weekly through Week 13.

1. Food consumption: During the first four weeks of the study, fresh food was added on a weekly basis. Because of apparent food spoilage due to contamination by feces and urine, males and females in the 800 ppm groups and females in the 400 ppm group exhibited decreased food consumption. The problem was remedied at Week 4 by adding fresh food midweek. During Week 4

food consumption for the high dose animals was significantly higher than the control value. No amendment was made to the study protocol, reflecting the change in feeding procedure.

NOTE: It is not clear to the reviewer why the food consumption data (Study Appendix 3) for cages 11, 17, 28 and 32 were not included in the statistical analyses. The footnote to the table in Appendix 3 states that the values for these cages were excluded because there were only one or two animals in the cage. Since food consumption was calculated as the amount of food consumed divided by the number of animals in the cage, the actual number of animals in the cage is irrelevant. This assumes, however, that food consumption was calculated on a prorated basis depending on the number of animals in the cage at a given time.

2. Food efficiency: Not determined

3. Compound Intake: Because of the above mentioned problems with food consumption, the compound intake values during the first four weeks of the study must be disregarded. The average compound intake for Weeks 5 through 13 is summarized in Table 4, below.

Table 4: Average Compound Intake (The reviewer calculated the means for Weeks 5 through 13 from data presented in Table 6 of the study)

Study Group	Compound Intake (mg/kg/day)	
	Male	Female
LDT	0.8	1.0
MDT1	8.1	10.9
MDT2	33.5	43.9
MDT3	67.2	73.2
HDT	141	153

2. Water consumption: Water consumption was determined during Weeks 1, 2, 3, 4, 8, and 12. Although some of the values appeared to achieve statistical significance, improper statistical procedures were used (See note in section I. E. Statistics above).

NOTE: Again, it is not clear to the reviewer why the water consumption data (Study Appendix 4) for cages 11, 17, 28 and 32 were not included in the statistical analyses.

7

E. Cholinesterase activity: Plasma, erythrocyte and brain cholinesterase activities were determined at terminal sacrifice. According to the study protocol, blood samples were pooled prior to determination of plasma and erythrocyte cholinesterase activities. Since there is no indication that equal aliquots of each of the pooled blood sample were used, the resulting values are meaningless. Brain cholinesterase data are summarized in Table 5.

Table 5: Brain Cholinesterase Activity (U/g tissue) (Data from Table 7 of the study)

Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
Male	8.56	12.24***	7.34*	1.75***	1.29***	1.11***
Female	8.28	8.56	5.90***	3.43***	1.34***	0.96***

* $p < 0.05$, *** $p \leq 0.001$

F. Hematology: Not performed.

G. Sacrifice and Pathology: Detailed gross pathological examinations were performed on animals sacrificed in moribund condition, dying during the study, or surviving to terminal sacrifices. All of the tissues listed below were fixed; selected tissues were examined histologically (X); other tissues (XX) were weighed before fixation. One-half of each brain was used for determination of cholinesterase activity, the other half was used for histological examination.

Digestive system
X Salivary glands
X Esophagus
X Stomach
X Duodenum
X Jejunum
X Ileum
X Cecum
X Colon
X Rectum
XX Liver
X Pancreas
X Gall bladder
X Tongue

Respiratory
X Lungs
X Trachea

Cardiovas./Hematol
XX Heart
X Aorta
X Lymph nodes
XX Spleen
X Thymus

Urogenital
XX Kidneys
X Urinary bladder
XX Testes
X Epididymides
X Prostate
X Seminal vesicle
X Ovaries
X Uterus
X Cervix

Neurologic
XX Brain
X Periph. nerve
X Spinal cord
X Pituitary
X Eyes/Optic nerve

Glandular
XX Adrenals
X Mammary gland
X Parathyroids
X Thyroids

Other
X Bone
X Skeletal muscle
X Skin
X Gross lesions
X Skull
X Harderian glands

8

1. Organ Weights: Sporadic significant differences in the absolute organ weights of both males and females were noted, however, the differences did not appear to be treatment-related. Organ weights relative to body weights showed significant elevation in the liver weights of females (Table 6).

Table 6: Relative Liver Weights for Females (Data summarized from Table 9 of the study)

Organ	CON	LDT	MDT1	MDT2	MDT3	HDT
Liver	5.13	5.41	5.48	5.69**	5.66*	5.90***

* $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

2. Gross Pathology: Gross pathological examination of animals dying or sacrificed in moribund condition during the study did no reveal any treatment-related effects.

3. Microscopic Pathology

a) Non-neoplastic lesions: The incidence data for non-neoplastic lesions are summarized in Table 7, below. Treatment-related effects included increased incidence of lipogenic pigmentation of the adrenal cortex and keratitis in females. Historical control data was not included with the study.

b) Neoplastic lesions: None noted.

III. DISCUSSION: This study evaluated the subchronic toxicity of test compound at dietary concentrations of 0, 5, 50, 200, 400 or 800 ppm (≈ 0, 0.8, 8.1, 33.5, 67.2 and 141 mg/kg/day (males); 0, 1.0, 10.9, 43.9, 73.2, and 153 mg/kg/day (females) respectively) when administered to mice for 13 weeks.

Table 7: Non-neoplastic Findings in Females (Data summarized from Table 11 of the study)

Observation	CON	LDT	MDT1	MDT2	MDT3	HDT
Adrenal cortex, lipogenic pigmentation	0/11	0/12	1/12	5/10	5/10	10/11
Eyes, keratitis	0/12	0/12	0/12	0/10	1/10	3/11

· $p \leq 0.05$, - $p \leq 0.01$, — $p \leq 0.001$

Because of problems noted above, the following parameters were considered to be invalid and therefore could not be evaluated:

1. Body weight data for the entire study

2. Food consumption data for first four weeks of the study.

3. Plasma and erythrocyte cholinesterase activities.

At terminal sacrifice, the following treatment-related effects were noted:

1. Brain cholinesterase was significantly lower in both males and females in the high dose group.

2. Females in the 200, 400 and 800 ppm dose groups had significantly higher relative liver weights.

3. Increased incidence of non-neoplastic lesions (lipogenic pigmentation of the adrenal cortex and keratitis) in high-dose females.

Because of the problems associated with the study, the reviewer felt that there was inadequate data to assign a NOEL and LOEL.

Classification: core - Supplementary (Can not be upgraded)

This study does not satisfy guideline requirements (82-1) for a subchronic oral toxicity study in mice.