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

SEP - 2 1988

006851

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: PYRINEX (Chlorpyrifos): A Thirteen Week Subchronic Toxicity Study in Rats, a Teratology Study in Rats, a Teratology Study in Rabbits and Three Mutagenic Studies.

FROM: Alan C. Levy, Ph.D. Alan C. Levy 8-29-88
Toxicologist, Review Section I
Fungicide/Herbicide Toxicology Branch/HED (TS-769C)

TO: Dennis Edwards - PM # 12
Registration Division (TS-767C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui 8/30/88
Section Head, Review Section I

and

William Burnam W. Burnam 9/1/88
Chief, Fungicide/Herbicide Toxicology Branch
Health Effects Division (TS-769C)

Registrant: Makhteshim-Agan (America) Inc.

Action Requested: Review a thirteen week subchronic toxicity study in rats, a teratology study in rats, a teratology study in rabbits and three mutagenic studies pertaining to PYRINEX (Chlorpyrifos)

Recommendation:

1. Toxicity in Dietary Administration to Rats for 13 Weeks (Study No. MAK/058/PYRA)

   Classification: Supplementary

2. Pyrinex, Teratogenicity Study in the Rat (Study No. MAK/101/PYR)

   Classification: Minimal

3. Pyrinex, Teratogenicity Study in the Rabbit (Study No. MAK/103/PYR)

   Classification: Supplementary

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4. Evaluation of Pyrinex in the Ames Mutagenesis Assay (Study No. 59487-AMKA)

   Classification: Acceptable

5. In Vitro Chromosomal Aberration Assay on Pyrinex (Chlorpyrifos) (Study No. 59487-CAA)

   Classification: Acceptable

6. CHO/HGPRT In Vitro Mammalian Cell Mutation Assay on Pyrinex (Chlorpyrifos) (Study No. 59487-CMA)

   Classification: Acceptable

Primary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy 8-29-88
Review Section V/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui
Section Head, Review Section V

I. Study Type: Teratology (rabbit)
(Guideline § 83-3)

Study Title: Pyrinex, Teratogenicity Study in the Rabbit

EPA Identification Numbers:

EPA Identification: 11678-UL
EPA Accession: 404364-08
EPA Record: 209807
Caswell: 219AA
Tox. Branch: 8-0425

Sponsor: Makhteshim-Agan (America), Inc.

Testing Laboratory: Life Science Research Israel Ltd. (LSRI)
PO Box 139, Ness Ziona 70541
Israel

Study Number: MAK/103/PYR

Study Date: July 15, 1987

Study Authors: Y. Rubin, Ph.D., A. Nyska, D.V.M. and T. Waner, B.V.Sc., M.Sc., MRCVS

Test Material:

Name: PYRINEX Technical (Chlorpyrifos)
Batch No.: 58009/9
Chemical Name: 0-0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothionate
Molecular Weight: 350.59
Appearance: Off-white granular crystals
Solubility: Insoluble in water; readily soluble in organic solvents such as acetone, carbon tetrachloride, carbon disulfide, methylene chloride, xylene and 1,1 trichloro-ethane.
Purity: 96.1%
Stability: Stable under normal storage conditions. Stable for several weeks in neutral or slightly acidic conditions when stored at room temperature.
VEHICLE: Maize oil

II. Materials and Methods

Animals

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Sexually mature virgin New Zealand HY/CR female rabbits, 4 to 5 months old and weighing 3.0-3.2 kg were obtained from Charles River Italia S.p.A. The animals were individually numbered and acclimatized for 15 days before mating. Males were of the same strain and source.

Temperature and humidity were regulated and the rabbits were exposed to a 14-hour light:10-hour dark cycle. A commercially pelleted rabbit diet (Altromin 2113) and drinking water (automatic watering system) were available ad libitum. Animals were caged individually.

Mating and Group Allocation

A table of random numbers was used to assign rabbits to experimental groups (before mating). Spontaneously estrous females were mated naturally with proven fertile males. After mating each female was administered 50 IU chorionic gonadotropin (Pregnyl, Organon) i.v. to ensure ovulation. The day of mating was defined as Day 0 of gestation. Each female was consecutively mated with two males (except for one control female which, according to data in Appendix 5, page 71 of the report, was mated with only one male). Each male was used to inseminate females from each group with the following exceptions: Nos. 206 and 207 not in control group and No. 207 not in 9 mg/kg/day group.

Test Material Formulation

The test material was formulated daily as solutions in maize oil.

Table 1

DOSING DESIGN FOR RABBIT TERATOLOGY STUDY WITH PYRINEX

Group	Test Material	Dosage (mg/kg/day)	Volume† (ml/kg/day)	No. of Animals
1	Vehicle Control	0	2	14
2	Pyrinex	1	2	14
3	Pyrinex	9	2	14
4	Pyrinex	81	2	21*
5	Pyrinex	140	2	14

* = Seven extra animals were added to this group because 3 rabbits (Nos. 801, 841 and 861) were erroneously administered the dose intended for Group 5 on Day 15 of gestation. [Study authors stated extra animals added so that overdosed rabbits might be excluded if exclusion was found to be warranted by the data. No apparent differences between the overdosed and regular-dosed Group 4 rabbits and therefore, maternal and fetal data were included in the group means.]

† = Volume administered orally

NOTE: Dosage is expressed in terms of the test material as received without correction for percent active ingredient.

These data are reproduced from a table on page 18 of the report.

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Doses for Groups 4 and 5 (81 and 140 mg/kg/day, respectively) were prepared by dissolving the required amount of test material in maize oil at 50-55°C. Doses for Groups 2 and 3 (1 and 9 mg/kg/day, respectively) were prepared by serial dilution of the Group 4 dose with maize oil. Concentration and stability (4 and 24 hours) of Pyrinex in the dosing solutions were measured once during the course of the study.

Table 2

PYRINEX IN DOSING SOLUTIONS - RABBIT TERATOLOGY

Group	Expected Concentration of Pyrinex		Analysis - mg/g (%)		
	mg/ml	mg/g*	Time 0	4 Hours	24 Hours
1	0	0	-	-	-
2	0.50	0.46	0.45(98)	0.45(98)	0.43(93)
3	4.50	4.14	4.39(106)	4.46(108)	4.24(102)
4	40.50	37.26	40.29(108)	40.52(109)	40.73(109)
5	70.00	64.40	77.66(121)	77.54(120)	75.94(118)

* = Assuming the weight of one ml of maize oil to be 0.92 g (British Pharmacopeia, 1980).
Data extracted from a Table on page 18 of the report as well as Appendix 4 of the report (pages 68-70). Percentages calculated from these data.
The analyses were performed by Analyst, Ltd., Kiryat Weizmann, Rehovot, Israel.
Peroxide values were also measured (Appendix 4) and were considered to be low (1.01 and 0.82).

Dosing

The rabbits were dosed by intragastric gavage from Days 7 through 19 of gestation. The dose administered daily to each rabbit was based on the animal's body weight on that day.

In addition to the three over-dosed rabbits referred to in the Table 1 footnote, one Group 4 animal (81 mg/kg/day) apparently was not dosed on one day (determined by post-dosing weighback of the dose container). It was not possible to determine which rabbit was not dosed nor on which day of gestation this occurred. The over-dosing occurred on Day-15 of gestation and as the higher dose group of 140 mg/kg/day did not appear to cause any developmental abnormalities, it would not be expected that treatment related changes would be observed due to the over-dose. Therefore, neither the over-dosing nor non-dosing were considered to affect the validity of the study results as: no apparent developmental abnormalities in the over-dosed dams; and, one non-dosed rabbit was a relatively small percent of the total number of dosed animals in this group (1/21 - 5%).

In-Life Observations

All rabbits were examined daily for signs of ill health or toxicity. Any rabbit found dead or sacrificed moribund was subjected to a gross necropsy in order to ascertain the cause of death.

Rabbits were weighed on Days 0, 3, 7-19, 22, 25 and 29 of gestation. Food consumed by each gestating female was measured twice weekly.

Plasma cholinesterase activity was measured once before mating and again after at least ten dosing days.

Terminal Data

Females were killed by injected Pentobarbitone Sodium on Day 29 of gestation. The following were recorded:

1. Macroscopic abnormalities of the reproductive tract.
2. Number of corpora lutea in each ovary.
3. Weight of gravid uterus.
4. Distribution of live and dead fetuses as well as distribution of resorption sites (classified as early or late) in each uterine horn.
5. Individual placental weight.
6. Individual fetal weight and crown-rump length.
7. External anomalies of individual fetuses.
8. Fetal examination (fetus killed by subcutaneous Pentobarbitone Sodium injection): contents of abdominal and thoracic cavities; sex recorded; skull sectioned transversely through frontal-parietal suture and brain examined; and skeletal (Alizarin red S staining).

Pre-implantation loss was calculated by:

$$\frac{\text{No. corpora lutea - No. implantations}}{\text{No. corpora lutea}} \times 100$$

Post-implantation loss was calculated by:

$$\frac{\text{No. implantations - No. live fetuses}}{\text{No. implantations}} \times 100$$

Descriptions of data calculations, statistical analyses and data presentation were included in the report.

A Quality Assurance statement was present.

The study protocol and amendments were included.

A copy of the Materials and Methods section from the report is appended. 6

The reviewer has the following comment regarding the Materials and Methods section: There are rabbits in each group which were said to be "not pregnant." Please indicate how this was determined.

III. Results

Clinical Signs and Mortality

There were no clinical signs which appeared to be attributable to Pyrinex administration.

There were four rabbits which died.

1. No. 857, Group 2, 1 mg/kg/day, found dead on Day 13. Few feces, diarrhea and blood on the tray under the cage. Necropsy showed severe diarrhea; duodenum, ileum and jejunum were empty with slight serous fluid contents; hemorrhagic foci in gastric mucosa. The study authors concluded that death was probably due to dehydration resulting from acute enteritis. The animal was pregnant.

2. No. 815, Group 4, 81 mg/kg/day, found dead on Day 18. Few feces and diarrhea. Necropsy showed left lung partially replaced by fibrous tissue; tracheal mucosa congested; abdominal cavity contained serous turbid exudate. The study authors concluded that death was probably due to lung dosing. The animal was pregnant.

3. No. 860, Group 4, 81 mg/kg/day, found dead on Day 8. Decreased motor activity and hypothermia. Necropsy showed thoracic cavity filled with blood-stained fluid; right lung was edematous with fibrous exudate over the heart. The study authors concluded that death was probably due to lung dosing. The animal was pregnant.

4. No. 843, Group 5, 140 mg/kg/day, presented for necropsy on Day 19. Few feces. Necropsy showed hemorrhagic contents of the trachea; edematous lungs; blood-stained contents of the abdominal cavity; fluid gastric contents with hemorrhagic gastric mucosa. The study authors concluded that death was probably due to pulmonary edema. The animal was pregnant. [Reviewer comment: Also, may have been due to lung dosing.]

Rabbit No. 849, Group 5, 140 mg/kg/day was killed after premature delivery on Day 28. The litter had 14 live or freshly dead fetuses and one identifiable late resorption. The authors concluded that: no important fetal malformation was found at necropsy or at skeletal evaluation of the fetuses; data for fetal weight and length confirm premature delivery; and necropsy examination did not reveal the cause of premature delivery.

Food Consumption

There did not appear to be a negative difference between treated and control groups regarding food consumption during the dosing period (Days 7-19) with the possible exception of the Days 15-19 period when the control mean $\pm$ was 140 ± 48 g/animal/day and the Group 5 (140 mg/kg/day) mean was 114 ± 54. During this interval 5/12 Group 5 rabbits ate less than 100 g/day compared with 2/13 from the control group. In addition, after the cessation of dosing, the controls ate a group mean of about 180 g/day and the Group 5 rabbits ate a group mean of about 219 g/day. Prior to dosing, the control mean was about 192 g/day and for Group 5, 224 g/day.

Body Weight

Table 3 shows group mean body weights for reported weighing days during gestation as well as corrected body weight (minus uterine weight) and body weight gains for the following intervals: entire period of gestation, prior to dosing, period of dosing and period from cessation of dosing until sacrifice on Day 29. The only apparent difference between treated and control values was a group mean weight loss during the dosing interval (Days 7-19) in the 140 mg/kg/day dose only. Post-dosing, there was a compensatory increase in weight gain in this group, so that the weight gain during gestation (total or corrected body weight gain) was similar for the control and all four Pyrinex groups. It therefore appears that the 140 mg/kg/day dose decreased body weight gain only during the period of dosing.

Plasma Cholinesterase

Plasma cholinesterase levels, before as well as after 10 days of dosing are presented in Table 4. A mean dose-response percent decrease of 57-72 was observed. It is noted that the "before commencement" group means range from 657.1 to 377.6 IU/l with Standard Deviations (S.D.) of 498.8 to 276.7. A review of individual animal data indicates that 12/13 controls had lower values after dosing than before. The 1 and 9 mg/kg/day individual values after dosing were lower than before by an even greater amount.

[There are no individual animal data for the 81 and 140 mg/kg/day groups included in the report. It appears that "typed" page number D-40 was omitted, although the hand-written page numbers (of a total of 208 pages) are in sequence. Utilizing the mean $\pm$S.D. group values and considering that the primary purpose of this study was to ascertain maternal and fetal effects of Pyrinex administered during gestation Days 7-19, it is felt that the omission of these individual cholinesterase values would not compromise the validity of the study and therefore shall not be requested.]

Table 4

PLASMA CHOLINESTERASE GROUP MEAN VALUES - RABBITS ADMINISTERED PYRINEX

Group	Dose (mg/kg/day)	Before Commencement (IU/l)	After 10 Doses (IU/l)	Percent Reduction
1	0	615.4$\pm$383.1 (13)*	451.5$\pm$274.7 (13)	-
2	1	377.6$\pm$276.7 (13)	196.4$\pm$57.0^c (13)	57
3	9	657.1$\pm$498.8 (13)	142.2$\pm$30.1^c (13)	69
4	81	571.1$\pm$394.7 (15)	136.2$\pm$29.6^c (10)	70
5	140	654.5$\pm$424.3 (12)	125.3$\pm$26.6^c (12)	72

* = Mean$\pm$Standard Deviation (No. of Rabbits)
c = Significantly different from control, $p<0.001$, Student's t-test.
Data extracted from Table 5, page 39 of the report.

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Table 3

MEAN BODY WEIGHTS DURING GESTATION OF RABBITS ADMINISTERED PYRINEX

Mg/kg/day	0	1	9	81	140
DAY POST COITUS	(13)[a]	(13)	(13)	(15)	(12)
0	3253±168[b]	3308±193	3378±162	3409±169	3329±154
3	3285±176	3386±166	3457±164*	3467±158**	3425±177*
7	3347±233	3431±211	3495±183	3502±157*	3497±223
8	3318±224	3379±224	3439±211	3458±184	3438±245
9	3279±228	3387±231	3455±214	3455±201*	3407±267
10	3292±228	3373±248	3453±232	3463±221	3418±253
11	3295±220	3389±238	3474±247	3469±228	3414±240
12	3352±234	3438±268	3529±260	3514±239	3466±267
13	3355±207	3448±240	3541±211*	3534±242*	3446±256
14	3405±224	3497±250	3576±193	3569±247	3449±270
15	3434±225	3538±249	3630±198*	3605±259	3473±274
16	3443±234	3540±249	3637±184*	3621±263	3476±291
17	3426±236	3553±258	3642±198*	3613±288	3457±293
18	3435±249	3570±236	3652±204*	3607±282	3454±294
19	3435±247	3586±242	3663±196*	3609±291	3398±267
22	3479±238	3642±238	3747±224**	3674±278*	3539±285
25	3586±242	3713±244	3817±220*	3771±258	3654±290 d
29	3692±223	3833±269	3904±240*	3862±283	3794±276 e
29[c]	3177±199	3277±249	3321±241	3306±210	3275±226 e
0-29 (B.W. chg.)	439±134	525±145	525±173	453±185	461±178 e
0-7	94±98	122±77	116±109	93±59	168±91[a]
7-19	88±176	155±76	168±104	107±167	-99±193[b]
19-29	257±82	242±91	241±110	253±81	401±120[c]e

a=Number of animals b=Mean±Standard Deviation c=Net of gravid uterus d=10 rabbits
e=11 rabbits B.W. chg.=Body Weight change
Statistical Significance from control, Student's t-test; p<: *=0.05; **=0.01; ***=0.001
Data extracted from Table 4, pages 36-38 of the report.

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Fetal Observations at Necropsy

Litter data from Cesarean section on Day-29 of gestation are presented in Table 5 (a photocopy of Table 6, page 40 of the report). According to the study authors, post-implantation loss was significantly ($p<0.001$) higher than the control value in Groups 3 and 5 (9 and 140 mg/kg/day, respectively) when Freeman-Tukey arcsine transformed data were compared under parametric assumptions. Neither group was significantly higher than controls when tested non-parametrically, using rank order methods. The authors further stated that the absence of a similar finding in Group 4 (81 mg/kg/day) indicated that at least in Group 3, this observation was due to chance variation and, "should not be ascribed to treatment". A review of individual litter data (Appendix 10, pages 106-114 of the report) indicated that post-implantation loss of at least 2 resorptions/dam occurred in the following number of litters (mg/kg/day): 0 = 2/13 (15%); 1 = 4/13 (31%); 9 = 5/13 (38%-one had 12 resorptions); 81 = 3/15 (20%); and 140 = 6/11 (55%). Therefore, a suggestion exists that administration of Pyrinex may cause an increase in the number (%) of litters with post-implantation loss.

Mean fetal weights in the 140 mg/kg/day group were stated by the authors to be less (not significant to $p<0.05$) than control (40.7 $\pm$6.0 vs 45.4$\pm$5.7). The mean data are seen in Table 5. A review of individual fetal body weight data (Appendix 11, pages 118-150 of the report) indicates that numbers of fetuses weighing less than 40 g/total number of fetuses in a group were as follows (mg/kg/day): 0 = 29/108 (27%); 1 = 42/117 (36%); 9 = 51/126 (40%); 81 = 38/128 (30%); and 140 = 45/76. (59%, does not include premature delivery). In addition, 4 fetuses in the 81 mg/kg/day litter No. 873 weighed 22.3-23.5 g (plus 3 fetal weights not recorded). The other groups did not have any litters with predominantly "light" (under 30 g) fetuses.

[Individual fetal data for Group 4 (81 mg/kg/day) litter No. 880 were not included in the report. These data should have appeared in Appendix 11, typed page No. D-80 (this page is missing) and between hand-written report page Nos. 143 and 144. On Table 5 (Table 6, page 40 of the report), Group 4, Mean fetal weight and SD, the N = 14; whereas, the N for all other parameters is 15. The registrant is requested to explain/correct the value of N as well as supply the individual fetal data for the above mentioned litter No. 880 (typed page No. D-80).]

Crown-rump length appears to be shorter in the Group 5 (140 mg/kg/day) fetuses ($p<0.05$) compared with controls (Table 5). The numbers of fetuses with crown-rump length less than 90 mm are as follows (mg/kg/day): 0 = 11/108 (10%); 1 = 18/117 (15%); 9 = 26/126 (21%); 81 = 15/128 (12%); and 140 = 24/76 (32%).

The following visceral/external findings were observed at Cesarean section:

0 mg/kg/day - One fetus with a diaphragmatic hernia and a second fetus in the same litter with a rudimentary tail.

1 mg/kg/day - No remarkable findings.

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9 mg/kg/day - One fetus with a major circulatory malformation (agenesis of the interventricular cardiac septum, rudimentary pulmonary artery and enlarged aortic arch); two additional fetuses from the same

Table 5

Group mean litter data on day 29 of gestation

Group		1	2	3	4	5
Test material				PYRINEX		
Dosage (mg /kg/day)		0	1	9	81	140

Group		Corpora lutea	Weight of gravid uterus (g)	Live fetuses			Resorptions			Implantation loss pre- (%)*	Implantation loss post- (%)*	Mean fetal wt. and SD (g)	Mean CRL and SD (mm)	Mean placental wt. and SD (g)
				M	F	Total	Early	Late	Total					
1	i	10.4	515.5	3.8	4.5	8.3	0.1	0.4	0.5	15.2	6.5	45.4	97.4	6.0
	ii	1.4	99.0	1.9	1.5	2.1	0.3	0.8	0.8	4.2	2.2	5.7	4.2	1.4
	iii											5.7	5.5	1.0
	N	13	13	13	13	13	13	13	13	13	13	13	13	13
2	i	11.1	556.4	4.1	4.9	9.0	0.3	0.5	0.8	13.1	8.6	44.3	95.9	6.0
	ii	2.1	109.3	1.7	1.7	2.4	0.5	0.7	0.9	3.4	2.5	4.7	4.3	0.7
	iii											5.6	4.7	0.9
	N	13	13	13	13	13	13	13	13	13	13	13	13	13
3	i	12.8b	582.8	4.9	4.8	9.7	1.2	0.7	1.9a	8.9c	13.8c	43.3	95.0	5.8
	ii	2.0	131.8	2.6	1.6	3.1	3.3	0.9	3.2	3.1	7.5	6.0	4.6	1.1
	iii											5.8	5.3	0.9
	N	13	13	13	13	13	13	13	13	13	13	13	13	13
4	i	11.9	555.6	4.7	4.7	9.5	0.4	0.4	0.8	11.9a	8.7	41.8	94.2	5.5
	ii	2.8	117.3	1.8	1.8	1.7	0.7	0.6	0.9	3.4	2.4	6.8	5.9	1.1
	iii											4.6	4.2	0.7
	N	15	15	15	15	15	15	15	15	15	15	14	15	15
5	i	12.1	518.5	4.9	4.1	9.0	0.7	1.0	1.7	11.5a	13.9c	40.7	93.2a	5.4
	ii	1.8	82.2	1.4	1.6	2.0	1.2	1.5	1.8	3.6	4.8	6.0	4.9	0.7
	iii											4.8	5.2	1.0
	N	11	11	11	11	11	11	11	11	11	11	11	11	11

* Freeman - Tukey arcsine transformed data.

i Group mean
ii Stardard deviation
iii Pooled weighted within - litter standard deviation

M male F female CRL crown - rump length

a: Significantly different from control, $p<0.05$, Student's t-test
b: Significantly different from control, $p<0.01$, Student's t-test
c: Significantly different from control, $p<0.001$, Student's t-test

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litter showed abnormal flexure (arthrogryposis) of the forelimb; and another fetus showed hydrocephalus, abnormally small lungs and no visible dentition.

81 mg/kg/day - Two fetuses with discrete areas of opacity in the lens (microscopic examination revealed a bilateral nuclear cataract in one fetus but no detectible abnormality in the other).

140 mg/kg/day - One fetus showed an irregularly shaped thorax.

Findings at Skeletal Evaluation

Table 6 shows numbers and percent of fetuses with skeletal changes (observations are included in this table only if at least 3 fetuses in any group were observed to have the finding). Table 6 indicates the numbers and mean percent of litters with skeletal changes (observations are included in this table only if more than one litter in a group possessed the finding).

There was a suggestive increase ($p<0.05$) in the number and percent of fetuses in the 140 mg/kg/day group which were reported to have unossified 5th sternebra and/or xiphisternum. In addition, the number of fetuses with irregular ossification of sternebrae 1-4 appeared to be greater than controls ($p<0.05$) in the 9 and 81 mg/kg/day dose groups, but not at 140 mg/kg/day. Reduced or incomplete ossification of the hyoid bone appeared to be more prevalent in fetuses from Pyrinex treated dams ($p<0.05$-0.001) than in controls.

The control fetus with a diaphragmatic hernia also had scoliosis with cervical verbetrae 4 and 6 as well as thoracic vertebrae 3-5 having only one hemicentrum. The fetus with a rudimentary tail had fusion of caudal vertebrae 2-6 with no caudal vertebrae distal to No. 6.

Scoliosis was observed in one 9 mg/kg/day fetus (agenesis of the right vertebral arch of the second cervical vertebra).

The 140 mg/kg/day fetus with the irregularly shaped thorax had the following skeletal changes: irregular bony projections and distal thickenings on various ribs with one rib slightly shortened; lumbar vertebral arches on the right side were slightly larger than those on the left (resulting in scoliosis); and multiple irregular centers of ossification were present (bilaterally) lateral to lumbar vertebrae 6 and 7.

IV. Discussion

Analyses of the dosing solutions indicated from 93-121% of the expected concentration. These values are considered to be acceptable.

Table 6

SKELETAL OBSERVATIONS - FETUSES/LITTERS EXAMINED - RABBITS ADMINISTERED PYRINEX

mg/kg/day	0	1	9	81	140
No. Fetuses/Litters Examined	108/13	117/13	126/13	142/15	99/11
SKULL					
Reduced or incomplete ossification of interparietal bone	3 (2.8)[a]	4 (3.4)	3 (2.4)	5 (3.5)	2 (2.0)
	2 (3.1)[b]	3 (3.5)	3 (2.4)	3 (3.1)	2 (1.7)
Intraparietal bone unossified	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	3 (3.0)
	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	2 (3.2)
Interparietal-supraoccipital suture open	8 (7.4)	2 (1.7)*	7 (5.6)	4 (2.8)	11 (11.1)
	5 (6.9)	2 (1.7)	4 (4.9)	3 (3.5)	6 (10.3)
Reduced or incomplete ossification hyoid bone	2 (1.9)	10 (8.5)*	17 (13.5)***	16 (11.3)**	8 (8.1)*
	1 (1.4)	7 (8.5)*	8 (12.2)**	7 (10.3)*	5 (7.5)
Hyoid cornu angulated	3 (2.8)	6 (5.1)	2 (1.6)	4 (2.8)	1 (1.0)
	2 (2.6)	5 (5.3)	2 (1.3)	4 (2.7)	1 (0.8)
SPINAL COLUMN & THORAX					
13th (lumbar) rib present bilaterally	71 (65.7)	73 (62.4)	79 (62.7)	83 (58.5)	77 (77.8)
	13 (67.1)	13 (63.6	13 (63.2)	15 (58.4)	11 (78.8)
13th (lumbar) rib present unilaterally	11 (10.2)	16 (13.7)	12 (9.5)	21 (14.8)	9 (9.1)
	5 (9.5)	8 (13.9)	6 (8.2)	9 (13.8)	6 (9.2)
Irregular ossification one or more stern. 1-4	1 (0.9)	2 (1.7)	7 (5.6)*	8 (5.6)*	1 (1.0)
	1 (0.7)	2 (1.8)	6 (8.7)	3 (5.9)	1 (0.9)
Two or more sternebrae fused or connected	1 (0.9)	1 (0.9)	3 (2.4)	0 (0.0)	0 (0.0)
	1 (1.0)	1 (1.0)	3 (2.1)	0 (0.0)	0 (0.0)
Bony plaque adjacent to sternebra	10 (9.3)	6 (5.1)	2 (1.6)**	3 (2.1)*	1 (1.0)**
	3 (7.8)	5 (6.0)	2 (1.3)	3 (2.0)	1 (1.3)
Irregular ossif. 5th stern. &/or xiphisternum	12 (11.1)	19 (16.2)	16 (12.7)	27 (19.0)	19 (19.2)
	5 (9.6)	8 (14.2)	7 (15.2)	12 (17.6)*	8 (17.3)
5th sternebra &/or xiphisternum unossified	1 (0.9)	1 (0.9)	2 (1.6)	3 (2.1)	6 (6.1)*
	1 (0.9)	1 (1.0)	1 (1.3)	1 (1.8)	3 (5.0)
APPENDICULAR SKELETON					
Reduced ossification of long bone epiphyses	15 (13.9)	26 (22.2)	24 (19.0)	44 (31.0)**	19 (19.2)
	6 (13.1)	8 (20.6)	9 (16.1)	12 (29.4)	9 (18.7)
Ilium articulating with 1st or 1st+2nd sacral vertebra (bilaterally)	33 (30.6)	37 (31.6)	54 (42.9)	55 (38.7)	31 (31.3)
	9 (30.7)	13 (31.5)	10 (40.2)	15 (39.3)	8 (28.0)
Ilium articulating with 1st or 1st+2nd sacral vertebra (unilaterally)	1 (0.9)	8 (6.8)	5 (4.0)	4 (2.8)	5 (5.1)
	1 (1.1)	6 (6.0)	3 (3.4)	3 (2.7)	5 (5.7)
< 4 distal phalanges ossified on 1 or both pedes	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.8)	2 (2.0)
	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.0)	1 (1.8)
< 5 distal phalanges ossified on 1 or both manus	0 (0.0)	0 (0.0)	1 (0.8)	3 (2.1)	0 (0.0)
	0 (0.0)	0 (0.0)	1 (0.6)	1 (2.2)	0 (0.0)

NOTE: Number and % fetuses with observation included in table only if at least 3 fetuses in any group were affected.

Significant difference from control, Fisher exact test (p<): *=0.05, **=0.01, ***=0.001

a = Number and (percent) of affected fetuses
b = Litter distribution of affected fetuses: number and (mean %)

Data extracted from Tables 9 and 10, pages 48-59 of the report.

Food consumption appeared to be slightly affected during dosing Days 15-19 with data showing not only a lower group mean in the 140 mg/kg/day rabbits, but also a somewhat larger number of animals having eaten less than 100 g/day. This dose of Pyrinex is considered to have a slight effect on food consumption during this time interval.

Test article administration at 140 mg/kg/day appeared to cause a slight body weight loss during the dosing period, but the rabbits compensated by gaining more weight than the control group post-dosing. Terminal body weights for all groups were essentially the same as were corrected final body weights (minus uterine weight) and body weight change during the entire period of gestation. Pyrinex is therefore considered to have a negative effect on this parameter only during the period of dosing.

Pyrinex administration caused a decrease in plasma cholinesterase levels after 10 doses. The lowest dose of 1 mg/kg/day resulted in a 57% reduction with the higher doses (9, 81 and 140 mg/kg/day) having about an equal effect (69, 70 and 72%, respectively).

Post-implantation loss appeared to be greater ($p<0.001$) than controls in rabbits dosed with 9 or 140 mg/kg/day, but not with 81 mg/kg/day. As indicated in the "Results" section, the study authors felt that the 9 mg/kg/day results should not be attributed to treatment. Post-implantation loss (at least 2 resorptions/dam) occurred in the following numbers (%): 2/12 (15%), 4/13 (31%), 5/13 (38%), 3/15 (20%) and 6/11 (55%) in the 0, 1, 9, 81 and 140 mg/kg/day groups. Therefore, there is the suggestion that this observation was caused by test article administration, at least in the 140 mg/kg/day group. No historical control data for this parameter were provided in the report.

Fetal weights and crown-rump lengths appeared to be slightly reduced in the 140 mg/kg/day group. The percent of fetuses in this group which weighed less than 40 g was about double the control value. The percent of fetuses exposed to 140 mg/kg/day with crown-rump lengths <90 mm was about 3 times greater than the control mean. Therefore, the possibility exists that the 140 mg/kg/day did have an effect on the size of fetuses. No historical control data for this parameter were provided in the report.

There were no gross changes observed at Cesarean section which appeared to be attributable to Pyrinex administration.

All dosed groups appeared to have higher incidences of reduced or incomplete ossification of the hyoid bone. Compared with historical data from the report (Table 7), the current control values are low and the treated values fall within historical limits (49% litter incidence). It is therefore unlikely that this finding is due to Pyrinex administration.

(14)

Instances of unossified 5th sternebra and/or xiphisternum appeared to be greater in the 140 mg/kg/day group than in the control. It is possible that this finding is related to the slightly smaller fetuses observed. The greater incidence of irregular ossification of sternebrae 1-4 in the 9 and 81 mg/kg/day groups only (control and 140 mg/kg/day were about the same), is not likely due to Pyrinex treatment. No other observed skeletal changes were considered to have been due to treatment.

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Table 7

HISTORICAL CONTROL INCIDENCE OF REDUCED OR INCOMPLETE OSSIFICATION OF THE HYOID BONE IN HY/CR NZW RABBITS AT LSRI (August 1984 - June 1987)

Study	Fetuses			Litters			Mean % of Affected Fetuses/Litter
	N	Affected	%	N	Affected No.	%	
A	124	8	6.5	14	4	29	5.6
B	123	34	27.6	14	13	93	27.2
C	97	4	4.1	12	3	25	10.5
D	112	5	4.5	13	5	38	4.3
E	108	21	19.4	13	9	69	19.0
F	117	32	27.4	13	9	69	26.2
G	135	16	11.9	15	8	53	11.8
H	156	14	9.0	16	7	44	8.9
I	131	6	4.6	14	3	21	3.7
MEAN	122.6	15.6	12.8	13.8	6.8	49.0	13.0

Data reproduced and calculated from Addendum 3, page 208 of the report.

V. Conclusions

The Cholinesterase Maternal No Observed Effect Level (NOEL) was not attained as the lowest dose tested (LDT), 1 mg/kg/day, caused a statistically significant increase in plasma cholinesterase inhibition. The Cholinesterase Maternal Lowest Observed Effect Level (LOEL) was 1 mg/kg/day (LDT).

The Maternal Systemic Toxicity NOEL and LOEL will be determined after review of the Registrant's response to the Agency's request for information. There was a decrease in food consumption (gestation Days 15-19) and a body weight loss (during the dosing period) followed by a compensatory weight gain in the 140 mg/kg/day group.

The Developmental Toxicity NOEL and LOEL will be determined after review of the Registrant's response to the Agency's request for information.

VI. Core Classification: Supplementary

This study may be upgraded depending upon the Registrant's response to the Agency's request.

Maternal Cholinesterase NOEL = Not attained
Maternal Cholinesterase LOEL = 1 mg/kg/day (LDT)

The Registrant is requested to provide the following information:

1. There are rabbits in each group which were said to be "non-pregnant". Please indicate how "non-pregnancy" was determined. Necropsy sheets for "non-pregnant" animals should be submitted. 15

2. Explain the value of N on Table 5 of this review, Group 4, mean fetal weight and SD where the value is 14 and for all other parameters N=15 (Table 6, page 40 of the report).

3. Individual fetal data for litter No. 880 (Group 4, 81 mg/kg/day) as this is missing from the report (Appendix 11, typed page No. D-80 is missing).

CHLORPYRIFOS

TXR 006851

Page____ is not included in this copy.

Pages 17 through 25 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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Primary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy 8-29-88
Review Section V/HED (TS-769C)

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T. Quang Bui
Section Head, Review Section V

I. Study Type: Teratology (rat)
(Guideline § 83-3)

Study Title: Pyrinex, Teratogenicity Study in the Rat

EPA Identification Numbers:

EPA Identification: 11678-UL
EPA Accession: 404364-07
EPA Record: 209807
Caswell: 219AA
Tox. Branch: 8-0425

Sponsor: Makhthshim-Agan (America), Inc.

Testing Laboratory: Life Science Research Israel Ltd. (LSRI)
PO Box 139, Ness Ziona 70541
Israel

Study Number: MAK/101/PYR

Study Date: July 15, 1987

Study Authors: Y. Rubin, Ph.D., N. Gal, M.Sc., T. Waner, B.V.Sc., MRCVS and A. Nyska, D.V.M.

Test Material:

Name: PYRINEX Technical (Chlorpyrifos)
Batch No.: 58009/9
Chemical Name: 0-0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothionate
Molecular Weight: 350.59
Appearance: Off-white granular crystals
Solubility: Insoluble in water; readily soluble in organic solvents such as acetone, carbon tetrachloride, carbon disulfide, methylene chloride, xylene and 1,1 trichloroethane.
Purity: 96.1%
Stability: Stable under normal storage conditions. Stable for several weeks in neutral or slightly acidic conditions when stored at room temperature.

VEHICLE: Maize oil

II. Materials and Methods

Animals

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Sexually mature virgin female CD rats were obtained from Charles

River Breeding Laboratories (U.K.) Ltd. Animals arrived in two body weight ranges (48-54 days old, 151-166 g and 55-65 days old, 182-203 g) in order to provide rats of comparable starting body weight over a mating period of approximately two weeks. There was a ten day acclimation period before pairing with stock males of the same strain and source. Temperature and humidity were regulated. A commercial rodent breeding diet (Altromin 1314) and drinking water (polyethylene bottles and stainless steel sipper-tubes) were available ad libitum. Animals were housed individually in high density polypropylene cages with stainless steel grid floors and lids.

Mating and Group Allocation

Females were paired one-to-one with males. On the morning after pairing the paper under the tray was examined for copulation plugs. If a plug was found a vaginal smear was prepared and a semi-quantitative estimate of sperm was made. Males were re-used for subsequent matings after a minimum 2-day rest. Mated females were assigned to groups in rotation (in order of mating) except that assignment may have been altered in order to ensure that no individual male contributed disproportionately to any one group and that the Day 0 body weight distribution was similar among all groups. Day 0 of gestation was the day on which copulation plugs and sperm-positive vaginal smears were found.

Test Material Formulation

The test material was formulated daily as solutions in maize oil.

Table 1

DESIGN FOR RAT TERATOLOGY STUDY WITH PYRINEX

Group	Test Material	Dosage (mg/kg/day)	Volume† (ml/kg/day)	No. of Animals
1	Vehicle Control	0.0	5	32
2	Pyrinex	0.5	5	32
3	Pyrinex	2.5	5	32
4	Pyrinex	15.0	5	32

† = Volume administered orally.

Data reproduced from a table on page 17 of the report.

Table 2

PYRINEX IN DOSING SOLUTIONS - RAT TERATOLOGY

Group	Expected Concentration of Pyrinex mg/ml	mg/g	[mg/gm][a]	Analysis - mg/g (%) [%][a] Time 0	4 Hours	24 Hours
1	0.0	0.0	0.0	none	none	none
2	0.1	0.1	0.09	0.09 (90)[100]	0.09 (90)[100]	0.09 (90)[100]
3	0.5	0.5	0.46	0.47 (94)[102]	0.48 (96)[104]	0.45 (90)[98]
4	3.0	3.0	2.76	2.96 (99)[107]	2.97 (99)[108]	2.82 (94)[102]

a = Added by reviewer. Assuming the weight of one ml of maize oil to be 0.92 g (British Pharmacopeia, 1980. Stated in rabbit teratology report.)
Data extracted from Appendix 4, page 61 of the report. Percentages calculated from these data.
The analyses were performed by Analyst, Ltd., Kiryat Weitzmann, Rehovot, Israel.
Peroxide values were also measured (Appendix 4, page 62): 0.99 and 1.13 for a mean of 1.06.

Dosing

The rats were dosed by intragastric gavage from Days 6 through 15 of gestation. The dose administered daily to each rat was based on the animal's body weight on that day.

In-Life Observations

All animals were examined daily for signs of toxicity. Rats were weighed on Days 0, 3, 6-15, 17 and 20 of gestation. Food consumed by each gestating female was measured twice weekly.

Plasma cholinesterase levels were measured from 10 mated females (satellite group) per group on Day 15 of gestation and on 10 rats from each group immediately prior to necropsy on Day 20. Ether was used as the anesthesia and bleeding was via the retrobulbar sinus.

Terminal Data

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Females were killed by carbon dioxide between 8:30 and 12:30 on Day 20 of gestation. The order of sacrifice each day was such that the mean time of sacrifice was approximately equal for all groups. The

following were recorded:

1. Macroscopic abnormalities of the reproductive tract.
2. Number of corpora lutea in each ovary.
3. Weight of gravid uterus.
4. Distribution of live and dead fetuses as well as distribution of resorption sites (classified as early or late) in each uterine horn.
5. Individual placental weights.
6. Individual fetal weight, crown-rump length and sex.
7. External anomalies of individual fetuses.
8. Fetal examination: thoracic and abdominal contents of about one-half of each litter were dissected and examined; after evisceration these fetuses were fixed in ethanol for skeletal staining (Alizarin red S) and evaluation; and the remaining fetuses (about one-half of each litter) were fixed in Bouin's fluid for free-hand sections (Wilson technique) and examination of visceral organs.

Pre-implantation loss was calculated by:

$$\frac{\text{No. corpora lutea - No. implantations}}{\text{No. corpora lutea}} \times 100$$

Post-implantation loss was calculated by:

$$\frac{\text{No. implantations - No. live fetuses}}{\text{No. implantations}} \times 100$$

Descriptions of data calculations, statistical analyses and data presentation were included in the report.

A Quality Assurance statement was present.

The study protocol and amendments were included.

A copy of the Materials and Methods section from the report is appended.

There are no comments regarding the Materials and Methods section.

III. Results

Clinical Signs and Mortality

There was no mortality.

A single subcutaneous mass observed during the in-life phase of the study was retained at necropsy for histopathological evaluation. (29)

The only observed clinical sign which was felt to be possibly due to Pyrinex administration was tremor in 3 high-dose (15 mg/kg/day) rats. This was noted on or one day after the last day of dosing, suggesting a cumulative effect.

Food Consumption

There was a slight, though statistically significant ($p<0.01$), decrease in the group mean food consumption of the 15 mg/kg/day females when compared with the control value during gestation days 7-9 (Table 3). A review of individual values (Appendix 7, pages 75-82 of the report) substantiates this relatively small decrease.

Table 3

MEAN FOOD CONSUMPTION FOR RATS RECEIVING PYRINEX DURING GESTATION

Group	Dose (mg/kg/day)		Gestation days 1-3	4-6	7-9	10-13	14-16	17-20
1	0	MEAN†	23.9	25.5	22.5	23.2	23.9	28.5
		SD	3.4	2.8	3.2	3.2	3.7	3.1
		N	32	32	31a	31a	22	22
2	0.5	MEAN	23.4	25.4	21.9	23.7	23.4	28.6
		SD	2.5	2.3	2.4	2.5	1.8	2.7
		N	31††	31	31	31	21	21
3	2.5	MEAN	23.9	25.2	22.0	23.3	23.1	28.4
		SD	2.6	2.7	2.7	2.6	1.7	3.7
		N	32	32	32	32	22	22
4	15.0	MEAN	23.9	25.0	20.2**	22.4	22.6	27.8
		SD	2.2	2.0	3.4	2.9	3.2	2.8
		N	31††	31	31	31	21	21

† = Grams of food/rat/day
†† = One rat not pregnant; data excluded from mean.
a = Apparent data recording error for one rat; data excluded from mean.
** = Significantly different from control, $p<0.01$, Student's t-test.
Data reproduced from Table 2, page 31 of the report.

Body Weight

Mean group body weights $\pm$ standard deviations are presented in Table 4. There were statistically significant lower body weights in the 15 mg/kg/day group ($p<0.01$ or 0.001 from Day 8 through 17 and $p<0.05$ at Day 20 or for terminal corrected weight [minus gravid uterine weight]). After Pyrinex administration (Days 6 through 15) body weight gain through Day 20 was about the same for all groups (difference between group mean body weights from Days 15-20 was 72.8, 72.9, 73.9 and 72.3 g for 0, 0.5, 2.5 and 15 mg/kg/day, respectively).

Plasma Cholinesterase Activity

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On Day 15 of gestation, plasma cholinesterase levels (Table 5) in the three Pyrinex groups were significantly ($p<0.001$) reduced from the control level (reduction of 51, 81 and 94% for the 0.5, 2.5 and

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Table 4

MEAN BODY WEIGHTS DURING GESTATION OF RATS ADMINISTERED PYRINEX

Mg/kg/day	0	0.5	2.5	15
DAY POST COITUS				
0 ------	231.8±9.4[a] (32)[b]	232.2±9.0 (31)	230.7±11.1 (32)	231.0±9.3 (31)
3 ------	245.3±11.8 (32)	246.2±12.0 (31)	243.3±12.4 (32)	244.5±9.9 (31)
6 ------	254.8±12.8 (32)	255.2±13.7 (31)	252.2±14.2 (32)	252.7±10.5 (31)
7 ------	257.7±11.7 (32)	258.6±12.6 (31)	255.9±13.3 (32)	254.5±10.2 (31)
8 ------	261.3±12.1 (32)	262.4±13.4 (31)	260.2±13.6 (32)	252.7±10.4**(31)
9 ------	265.9±13.2 (32)	266.9±14.0 (31)	264.3±13.7 (32)	254.9±11.4**(31)
10 ------	271.3±12.7 (32)	273.4±14.0 (31)	270.4±13.5 (32)	259.8±11.4***(31)
11 ------	275.9±13.1 (32)	277.9±15.0 (31)	275.5±13.8 (32)	265.4±10.9**(31)
12 ------	280.0±13.4 (32)	282.5±15.6 (31)	279.4±14.8 (32)	269.0±10.3**(31)
13 ------	284.7±14.1 (32)	286.7±16.1 (31)	284.3±15.5 (32)	271.8±9.5***(31)
14 ------	291.9±12.8 (32)	292.5±16.8 (31)	289.2±15.9 (32)	277.7±10.2***(31)
15 ------	298.0±15.6 (32)	301.6±17.0 (31)	298.1±16.7 (32)	283.7±10.6***(31)
17 ------	322.8±15.8 (22)	328.0±18.9 (21)	323.7±20.0 (22)	305.8±13.0**(21)
20 ------	370.8±17.4 (22)	374.5±26.2 (21)	372.0±24.2 (22)	356.0±15.9* (21)
20[c] -----	295.5±13.5 (22)	294.2±21.3 (21)	295.9±18.9 (22)	282.6±11.1* (21)
ADDED BY REVIEWER				
Days 6-15	43.2	46.4	45.9	31.0
6-17	68.0	72.8	71.5	53.0
15-20	72.8	72.9	73.9	72.3

a = Group mean ± standard deviation.
b = Number of rats in group.
c = Terminal body weight corrected (minus gravid uterine weight).
Statistically different from control, Student's t-test (p<): *= 0.05, **=0.01, ***=0.001

NOTE: There was one rat not pregnant in the 0.5 and 15 mg/kg/day groups; body weight values for these rats were not included in the means (number of rats in these groups are therefore 31 and then 21 rather than 32 and then 22).

Data extracted from Table 3, pages 32 and 33 of the report.

15 mg/kg/day groups, respectively). The Day 20 values (Day 15 was the last day of Pyrinex administration) indicated that only the 15 mg/kg/day animals had not returned to the control level (28% reduction, $p<0.01$ - Table 5).

Table 5

GROUP MEAN PLASMA CHOLINESTERASE ACTIVITY - RATS ADMINISTERED PYRINEX

Group	Dose (mg/kg/day)	Day 15 of Gestation	Day 20 of Gestation
1	0	913.01±151.93 (9)†	664.77±172.30 (10)
2	0.5	445.53±97.87*** (10)	594.26±140.55 (10)
3	2.5	174.28±97.30*** (10)	759.30±88.03 (10)
4	15.0	53.79±15.08*** (10)	480.74±117.74**(10)

† = Mean ± Standard Deviation (Number of samples) - IU/l
Statistically different from control, Student's "t" test:
** = $p<0.01$ *** = $p<0.001$
Data reproduced from Table 4, page 34 of the report.

Findings at Necropsy (Table 6 - Photocopy of Table 5, page 35 of the report)

Post-implantation loss was significantly greater ($p<0.01$) in the 15 mg/kg/day group (9.0±2.4% vs 7.0±2.1%). There were 8/22 control litters which had a loss of at least 7.0% vs 12/21 in the 15 mg/kg/day group.

Pre-implantation loss was significantly greater ($p<0.001$) in all three treated groups (mg/kg/day): 0 = 8.6±2.6%; 0.5 = 12.2±2.1%; 2.5 = 11.5±1.8%; and 15 = 11.4±2.7%). The number of litters in each group which had at least a 10.0% loss was as follows (mg/kg/day): 0 = 8/22 (36%); 0.5 = 13/21 (62%); 2.5 = 12/22 (55%); and 15 = 10/21 (48%).

The mean number of live male fetuses in the 15 mg/kg/day group was significantly ($p<0.05$) lower than controls (6.0±2.2 vs 7.6±1.8 for control). The total live litter size (male plus female) did not appear to be affected (13.4±1.7 vs 14.4±1.8 for control).

Group mean fetal weights and crown-rump lengths were significantly ($p<0.01$) greater in the 15 mg/kg/day group than in the control. The authors stated that the increased fetal weight did not correlate with low litter size ($r_s = 0.116$, Spearman rank correlation test).

Anomalies observed included: ablepheron (congenital absence of the eyelid) in one 2.5 mg/kg/day fetus; right aortic arch in one 15 mg/kg/day fetus; and a cranio-facial malformation with proboscis and anophthalmia in a 0.5 mg/kg/day fetus. None of these were considered to be treatment related.

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TABLE 6

Group mean litter data on day 20 of gestation

Group		1	2	3	4
Test material			PYRINEX		
Dosage (mg /kg/day)		0	0.5	2.5	15

Group	N	Corpora lutea	Weight of gravid uterus (g)	Live fetuses M	Live fetuses F	Live fetuses Total	Resorptions Early	Resorptions Late	Resorptions Total	Implantation loss pre- (%)*	Implantation loss post- (%)*	Mean fetal wt. and SD (g)	Mean CRL and SD (mm)	Mean placental wt. and SD (g)
1 i	22	17.1	75.3	7.6	6.8	14.4	1.0	0.0	1.0	8.6	7.0	3.33	36.0	0.48
ii		2.2	9.2	1.8	2.2	1.8	1.4	0.0	1.4	2.6	2.1	0.27	1.0	0.04
iii												0.28	1.5	0.06
2 i	21	18.2	80.3	8.0	7.0	15.0	0.9	0.0	0.9	12.2c	6.0	3.40	36.2	0.50
ii		2.4	9.1	2.1	1.8	1.7	1.1	0.2	1.1	2.1	1.8	0.18	0.8	0.04
iii												0.26	1.4	0.06
3 i	22	16.9	76.1	6.8	7.3	14.1	0.8	0.0	0.8	11.5c	5.8	3.44	36.3	0.49
ii		2.1	8.2	1.9	1.9	1.6	1.1	0.0	1.1	1.8	1.8	0.17	0.6	0.04
iii												0.25	1.3	0.06
4 i	21	17.0	73.4	6.0a	7.5	13.4	1.3	0.0	1.3	11.4c	9.0b	3.51b	36.7b	0.48
ii		2.6	10.1	2.2	1.9	1.7	1.5	0.0	1.5	2.7	2.4	0.25	0.9	0.05
iii												0.22	1.2	0.06

* Freeman - Tukey arcsine transformed data.

i Group mean
ii Stardard deviation
iii Pooled weighted within - litter standard deviation

M male F female CRL crown - rump length

a: significantly different from control, $p<0.05$, Student's t-test
b: significantly different from control, $p<0.01$, Student's t-test
c: significantly different from control, $p<0.001$, Student's t-test

Histopathological Findings

A firm subcutaneous mass observed during the in-life portion of the study on Day 20 of gestation (animal no. 3F75, 2.5 mg/kg/day) was found to be a malignant epithelial tumor.

Observations of Free-Hand Fetal Sections (Wilson Technique)

One 0.5 mg/kg/day fetus had anophthalmia and microphthalmia and one 2.5 mg/kg/day fetus had microphthalmia. Neither these nor any other findings were considered to be treatment related.

Skeletal Evaluation (Table 7)

There were no apparent adverse effects observed at fetal skeletal evaluation. Some statistically significant differences between the control and 15 mg/kg/day group were noted. These seemed to indicate a more advanced skeletal development in the treated group (mean fetal weight and crown-rump length were greater in the high-dose group than control - $p<0.01$).

IV. Discussion

Dosing solutions contained 90-99% (authors) or 98-108% (reviewer) of the expected amount of Pyrinex at the 0, 4 or 24 hour analyses. These values are considered to be acceptable.

The tremors noted in 3/21 15 mg/kg/day rats were considered to be related to Pyrinex administration. As the observations were made on days 15 or 16 of gestation (day 15 was the last day of dosing), the effect is regarded as being a cumulative one.

Regarding food consumption, the only difference between treated and control groups was an apparent ($p<0.01$) decrease observed in the 15 mg/kg/day group for the period of gestation days 7-9. This decrease is probably due to the initiation of Pyrinex administration on day 6. By the period of days 10-13, the animals had been acclimated to test material administration and appeared to eat approximately the same number of grams of food/day as did controls.

Body weights were significantly ($p<0.05$, 0.01 or 0.001) less in the 15 mg/kg/day group starting on gestation day 8 (dosing began on day 6) and continued throughout the study. However, after cessation of dosing (day 15), the gain in body weight was essentially the same in the three treated and the control groups. It therefore appears that this high dose of Pyrinex causes a decrease in body weight gain, but only during the period of test material administration.

(34)

There was a dose-response increase in plasma cholinesterase inhibition (51, 81 and 94%) as measured at day 15 of gestation. When values were determined on gestation day 20 (dosing days 6-15) the 0.5 and 2.5 mg/kg/day groups had levels similar to control, but there was still a

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Table 7

SKELETAL OBSERVATIONS - FETUSES/LITTERS EXAMINED - RATS ADMINISTERED PYRINEX

mg/kg/day	0	0.5	2.5	15
No. Fetuses/Litters Examined	161/22	157/21	158/22	146/21
SKULL				
Anterior fontanelle large	8 (5.0)*	5 (3.2)	3 (1.9)	0 (0.0)[b]
	4 (5.1)†	2 (3.3)	3 (1.9)	0 (0.0)
Anterior fontanelle small	0 (0.0)	1 (0.6)	1 (0.6)	5 (3.4)[a]
	0 (0.0)	1 (0.6)	1 (0.8)	3 (3.4)
Interparietal bone: reduced or incomplete ossification	51 (31.7)	38 (24.1)	36 (22.8)	28 (19.2)[d]
	15 (33.0)	16 (24.9)	13 (21.5)	12 (19.3)
SPINAL COLUMN AND THORAX				
14th (lumbar) rib present bilaterally	7 (4.3)	4 (2.5)	5 (3.2)	1 (0.7)[a]
	4 (4.0)	3 (2.3)	3 (2.9)	1 (0.5)
rib(s) angulated	5 (3.1)	0 (0.0)[a]	1 (0.6)	0 (0.0)[a]
	3 (3.3)	0 (0.0)	1 (0.6)	0 (0.0)
one or more sternebrae 1-4 unossified	11 (6.8)	2 (1.3)[b]	3 (1.9)[d]	0 (0.0)[c]
	6 (7.0)	2 (1.2)	2 (1.9)	0 (0.0)[af]
5th sternebra &/or xiphisternum unossified	106 (65.8)	99 (63.1)	102 (64.6)	77 (52.7)[d]
	20 (66.9)	19 (62.4)	21 (63.4)	19 (53.4)
APPENDICULAR SKELETON				
metacarpus v unossified bilaterally	116 (72.0)	113 (72.0)	98 (62.0)	80 (54.8)[e]
	21 (73.2)	21 (71.8)	19 (60.9)	18 (56.6)
1 or more metacarpi ii, iii, iv unossified (unilat. or bilat.)	7 (4.3)	1 (0.6)[a]	2 (1.3)	0 (0.0)[a]
	5 (4.5)	1 (0.7)	2 (1.2)	0 (0.0)[af]
pubic bone: reduced or incomplete ossification	11 (6.8)	9 (5.7)	4 (2.5)[a]	3 (2.1)[a]
	11 (7.0)	5 (6.0)	3 (2.5)[bf]	3 (2.0)[af]

* = Number and (percent) of affected fetuses.
† = Litter distribution of affected fetuses: number and (mean %).

Statistically different from control:
a = $p < 0.05$, Fischer exact test
b = $p < 0.01$, Fischer exact test
c = $p < 0.001$, Fischer exact test
d = $p < 0.05$, chi square test
e = $p < 0.01$, chi square test
f = $p < 0.05$, Mann-Whitney U-test

Data extracted from Tables 10 and 11, pages 43 - 52 of the report.

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28% reduction in the 15 mg/kg/day group mean. This observation appears to indicate that there is a residual cholinesterase inhibiting effect at the highest dose even after about 5 days after the last dose.

Post-implantation loss was slightly but significantly ($p<0.01$) greater in the 15 mg/kg/day group. This is considered to be a questionable chemical effect in light of the relatively small difference between 9% and 7% (group means). Historical study control means (Table 8) ranged from 4.4 ± 0.9 to 6.3 ± 1.8 (mean of 5.3 for 13 studies) indicating that the control and high-dose values are both above the historical values.

Pre-implantation loss was significantly ($p<0.001$) greater (and about equal) in all Pyrinex treated groups compared with the control. The number of dams with 2 or more resorptions was (mg/kg/day): 0 = 4/22 (18%), 0.5 = 6/21 (29%), 2.5 = 3/22 (14%), and 15.0 = 6/21 (29%) indicating that there is probably no difference between groups. Historical study control means (Table 8) ranged from 4.6 ± 1.0 to 17.0 ± 4.6 (mean of 10.6 for 13 studies). The three dose group means in this study were 12.2, 11.5 and 11.4% (with a control value of 8.6%) compared with 6/13 historical group means being at least 11.1. Therefore, when compared with historical data, the results in this current study do not seem to indicate a real difference between groups regarding pre-implantation loss.

There was no apparent effect of Pyrinex administration on litter size.

Mean fetal weights and crown-rump lengths were greater ($p<0.01$) in the 15 mg/kg/day group when compared with the control mean; however, the differences were slight (3.51 ± 0.25 vs 3.33 ± 0.27 g for fetal weight and 36.7 ± 0.9 vs 36.0 ± 1.0 mm for crown-rump lengths). The skeletal evaluation indicated the possibility of a slightly more advanced skeletal development. According to the study authors, this apparent increase in fetal weight did not correlate with a low litter size ($r_s = 0.116$, Spearmen rank correlation test). Historical study control means (Table 8) ranged from 3.24 ± 0.18 to 3.58 ± 0.23 g. There were 2/13 historical study mean values which were below the current study mean. In addition, historically, 1/13 had mean values above the 15 mg/kg/day dose value. It is therefore questionable that the apparent greater mean fetal weights are due to Pyrinex administration.

None of the anomalies (ablepheron, right aortic arch and a craniofacial malformation) were considered to be related to Pyrinex administration. Neither the anophthalmia and micropthalmia observed in a low-dose fetus or the microphthalmia in a mid-dose fetus were thought to be treatment related. There were no apparent adverse effects observed at skeletal evaluation. The differences noted between the 15 mg/kg/day and control fetuses seem to indicate a more advanced skeletal development in the treated group (Table 7).

Table 8

HISTORICAL CONTROL VALUES IN CD RATS AT LSRI (MARCH, 1984 TO OCTOBER, 1986)

Study	Pre-Implantation Loss (% mean±SD)	Post-Implantation Loss (% mean±SD)	Fetal Weight (g mean±SD)
A	11.1 ± 3.7	5.8 ± 1.1	3.45 ± 0.12
B	8.8 ± 2.1	4.7 ± 1.2	3.50 ± 0.21
C	17.0 ± 4.6	5.3 ± 1.9	3.58 ± 0.23
D	8.0 ± 2.9	5.7 ± 1.4	3.42 ± 0.25
E	11.2 ± 6.1	5.9 ± 1.1	3.53 ± 0.18
F	7.2 ± 2.6	4.4 ± 0.9	3.34 ± 0.21
G	16.4 ± 3.7	6.3 ± 1.8	3.24 ± 0.18
H	12.0 ± 3.3	4.8 ± 1.0	3.44 ± 0.26
I	10.1 ± 2.8	5.0 ± 1.1	3.47 ± 0.23
J	10.7 ± 3.7	5.0 ± 2.5	3.32 ± 0.22
K	4.6 ± 1.0	5.6 ± 1.2	3.49 ± 0.29
M	8.4 ± 1.9	6.1 ± 1.5	3.36 ± 0.32
N	11.9 ± 3.2	4.4 ± 1.2	3.46 ± 0.15
ADDED BY REVIEWER Means	10.6	5.3	3.43

DATA FROM REPORT

Pre-Implantation Loss: Addendum 3, report page 266.
Post-Implantation Loss: Addendum 4, report page 267.
Fetal Weight: Addendum 5, report page 268.

V. Conclusion

The Cholinesterase Maternal No Observed Effect Level (NOEL) was not attained as the lowest dose (0.5 mg/kg/day) caused a statistically significant increase in plasma cholinesterase inhibition. The Cholinesterase Maternal Lowest Observed Effect Level (LOEL) was 0.5 mg/kg/day. The Maternal Systemic NOEL was 2.5 mg/kg/day regarding a decrease in food consumption (during only the first few days of dosing) and a decrease in body weight gain (during the days of dosing). The Maternal Systemic LOEL was 15 mg/kg/day.

The Developmental Toxicity NOEL was 2.5 mg/kg/day (mid dose). The LOEL was 15 mg/kg/day where post-implantation loss occurred.

VI. Core Classification: Minimal

Cholinesterase Maternal Toxicity NOEL = Not attained
Cholinesterase Maternal Toxicity LOEL = 0.5 mg/kg/day (LDT)
Maternal Systemic Toxicity NOEL = 2.5 mg/kg/day
Maternal Systemic Toxicity LOEL = 15 mg/kg/day
Developmental Toxicity NOEL = 2.5 mg/kg/day
Developmental Toxicity LOEL = 15 mg/kg/day

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Primary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy
Review Section V/HED (TS-769C) 8-29-88

Secondary Reviewer: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q Bui
Section Head, Review Section V

I. Study Type: Subchronic Oral Toxicity (rodent): 90-day study (Guideline § 82-1)

Study Title: Toxicity in Dietary Administration to Rats for 13 Weeks [PYRINEX (Chlorpyrifos) Insecticide]

EPA Identification Numbers:

EPA Identification: 11678-UL
EPA Accession: 404364-06
EPA Record: 209807
Caswell: 219AA
Tox. Branch: 8-0425

Sponsor: Makhteshim-Agan (America), Inc.

Testing Laboratory: Life Science Research Israel Ltd. (LSRI)
PO Box 139, Ness Ziona 70541
Israel

Study Number: MAK/058/PYRA

Study Date: October 28, 1985

Study Authors: S. Crown, M.Sc., E. Gur, B.Sc., A. Nyska, D.V.M. and T. Waner, B.V.Sc., M.Sc., MRCVS

Test Material:

Name: PYRINEX TECHNICAL (Chlorpyrifos)
Batch No.: 760210
Chemical Name: O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothionate
Content of Active Ingredient (Chlorpyrifos): 95.5%
Appearance: Off-white granular cyrstals
Molecular Weight: 350.62
Solubility: Insoluble in water
Stability: Stable under normal storage conditions; decomposes above 160°C.

[The report stated that, "The Sponsor was responsible for the identity, concentration and purity of the test material received. Sponsor's analysis of the test material after termination of the study showed a concentration of 96% active ingredient."]

II. Materials and Methods

Animals

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Male and female Sprague Dawley rats about 4 weeks old and

weighing 52-83 gm were received from Charles River Breeding Laboratories, (U.K.) Ltd. The animals were acclimated for 10 days prior to treatment. The rats were housed 5 of one sex in a cage (high density polypropylene body, 56 x 38 x 18 cm, with stainless steel mesh floor and lid). Temperature, humidity and the light/dark cycle were regulated. Diet and water (polythene bottles with stainless steel sipper tubes) were supplied ad libitum.

Experimental Groups and Dosing

The animals were randomized into 4 experimental groups (control, 0.5, 10 and 200 ppm Pyrinex) using LSRI SOPs so that there were 20 rats/sex/group.

Pyrinex was melted in a waterbath. Six gm were added to 16 gm of sesame oil and preheated to 60°C to form 22 gm of mixture which was added to 150 gm of diet. This was stirred manually and mixed with 500 gm of diet for 10 minutes in a Kenweed mixer. The mixture was made up to 2, 3 and 4 kg diet (each amount mixed for 5 minutes in a Hobart vertical mixer). The mixture was then transferred to a second bowl and mixed for 15 minutes to provide a premix from which dietary concentrations were prepared by serial dilution. [The calculations in Figure 2, page 31 of the report were checked and appear correct.] The diet was prepared every two weeks with uneaten medicated feed measured and removed at the end of each week.

Analyses of dietary mixtures were performed by the Department of Analytical Chemistry, the Israel Institute of Biological Research, Ness Ziona, Israel. Homogeneity of Pyrinex was determined on 6 samples of premix, high-dose and mid-dose concentrations (no analytical methodology for low-dose concentration; serial dilution from higher concentrations was used to interpolate the validity of low-dose homogeneity). Stability in the diet was determined on high and mid concentrations from the Week 13 mix, 5, 13 and 26 days post-preparation. Animals received the dietary admix for a minimum of 13 weeks (until day of necropsy).

Observations, Body Weights and Food Consumption

Rats were examined twice daily (once/day on weekends and holidays) for mortality and clinical signs with appropriate recordings made. Animals were handled and closely examined at least once weekly (except Week 2 - no reason given in report). Body weights were measured for each rat on Day 1 of treatment and weekly thereafter. Food eaten per cage was calculated weekly from measurements of food given, food scattered and food remaining. Food conversion (bodyweight gain and food consumed) was calculated weekly. Achieved doses (mg/kg/day) were calculated weekly for each group and sex. Water consumption was assessed daily by visual examination of the bottles.

The eyes of all rats were examined by direct ophthalmoscopy prior to the start of treatment. Control and high-dose animals were also examined after Week 12.

Clinical Pathology

Ten males and 10 females from each group were selected prior to the start of the study. Blood was taken from the retro-orbital sinus (under ether anesthesia) after an overnight fast during week 13.

Hematology parameters examined were: hematocrit (packed cell volume - PCV), hemoglobin, erythrocyte count, leukocyte count, leukocyte differential and platelet count.

Blood chemistry parameters examined were: urea, creatinine, alkaline phosphatase (ALPH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (G-GTP), protein (total and differential), bilirubin (total), potassium, chloride, calcium, phosphorous, sodium and plasma cholinesterase.

Urine samples were collected during week 14 from individual metabolism cages during the period of 4 PM until 8:30 AM (no food or water) and the following parameters examined: volume, pH, specific gravity, glucose, protein, ketones, bilirubin, urobilinogen, blood pigments and sediment (microscopic examination).

Necropsy/Gross Pathology

After 13 weeks on the dietary admix, a necropsy was performed on all rats after carbon dioxide anesthesia and death. The clinical history was reviewed and the following examined: external surfaces and orifices as well as contents of the cranial, thoracic and abdominal cavities. The following organs were weighed (weights expressed as absolute and percent of body weight) from all groups: adrenals (left and right), brain, kidneys (left and right), liver, spleen and testes (left and right). About 40 tissues/rat were fixed in 4% formaldehyde (10% formalin) saline with the following exceptions: eyes - Davidson's fixative; bone marrow smears - methanol.

Histopathology

Microscopic examination was performed on about 35 tissues/animal (hematoxylin and eosin stain) from all rats in the control and high-dose groups plus abnormalities, kidneys, liver and lungs from the low- and mid-dose groups.

Descriptions of data calculations, statistical analyses and data presentation were included in the report.

A Quality Assurance statement was present.

The study protocol and amendments were included.

A copy of the Materials and Methods section from the report is appended.

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Comments regarding the Materials and Methods section are as follows:

This reviewer feels that the rats should have been housed 1/cage rather than 5/cage. Individual food consumption could only have been estimated. As food consumption was presented in the report as "g/rat", and especially in a dietary admix study, accuracy would have been increased if the animals had been individually housed.

III. Results

Homogeneity and Stability of Dietary Admix

Homogeneity values had a somewhat larger than expected range at Week 1 (1500 ppm pre mix = ±18%; 200 ppm = -15% & +22%; 10 ppm = ± 12%) but less of a range at Week 3 (1500 = ±2%; 200 = -3% & +5%; 10 = ±7%). Data from the Week 13 mix showed stability at the 1500, 200 and 10 ppm mixes.

Mortality and Clinical Signs

No mortality occurred during the study. Table 1 presents the incidence of clinical signs. No observations appeared to be due to Pyrinex administration.

Table 1

INCIDENCE OF SIGNS OBSERVED DURING TREATMENT OF RATS WITH PYRINEX BY DIETARY ADMIX FOR 13 WEEKS

	Males				Females			
Dose (ppm)	0	0.5	10	200	0	0.5	10	200
No abnormalities detected	16	16	14	14	16	18	17	15
OBSERVATIONS								
Orbital staining	2	3	5	2	0	0	0	1
Nasal staining	1	1	0	3	1	0	1	2
Hair loss	2	1	2	1	2	0	2	0
Wound	1	1	0	1	0	2	1	1
Urogenital staining	0	0	0	0	0	0	0	2
Hemorrhage (eye)	0	0	1	0	0	0	0	0
Swelling (eye)	0	0	0	1	0	0	0	0
Opacity	0	0	0	0	1	0	0	0

There were 20 rats/sex/group
Data extracted from Table 1, page 34 of the report.

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Body Weight

Body Weights are presented in Table 2 (a photocopy of Table 2, page 35 of the report). Mean body weight gains were lower (statistically significant from controls weeks 1-5 and 7) in the 200 ppm male group than in the control or other dose groups during the approximately first half of the study; but by week 13, the means were about the same for all male groups. In females, the 200 ppm group mean was below the control and other dosed groups means (statistically significant from control) during approximately the first three weeks. [No body weight gain data (group means or individual) were presented in the report.] Body weight gains, calculated by this reviewer, revealed no biological differences.

Food Consumption

Food consumption was measured/cage (5 rats) and expressed as grams consumed/rat/week. Table 3 shows the individual cage and group mean amount of food consumed. There did not appear to be any difference for either males or females between the low-dose (0.5 ppm) or mid-dose (10 ppm) groups and the control group. In the high-dose (200 ppm) animals there appeared to be an increase in food consumed with statistical significance in males at weeks 8, 9, 10 and 13 as well as in females during weeks 3-13.

Food Conversion and Achieved Dosages

Data were presented which indicated that the efficiency of food utilization (as measured by the food conversion ratio) was similar in all groups. Minimum and maximum achieved doses expressed as mg/kg/day were as follows: 0.5 ppm = 0.078 - 0.030; 10 ppm = 1.541 - 0.601; and 200 ppm = 30.677 - 13.053. As expected, achieved dosages decreased as the study progressed due to an increase in body weights accompanied by a relatively constant amount of food intake.

Hematology

Group means and standard deviations (SD) for hematology parameters are presented in Table 4. The only statistically significant differences between treated and control groups occurred in the 200 ppm males and females where a decrease was observed for packed cell volume (hematocrit), hemoglobin and red blood cells ($p<0.05$ or 0.01). A review of individual animal values indicated a strong possibility of a decrease.

[Appendix 8, page 90 of the report, Group 4M (200 ppm Pyrinex), lymphocytes, animal No. 68: the value is "1.33". This appears to be an error as the next lowest values are 8.7 and 8.8 with 8 of 11 values being 10.9 - 22.3.]

Blood Chemistry

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Table 5 shows the group mean values for all of the blood chemistry parameters examined. There was a statistically significant ($p<0.01$ or 0.001) reduction in cholinesterase levels in all three male dosed

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groups and in the mid- and high-dose female rats. [Female group means were 3300, 3639, 295 and 1427 IU (0, 0.5, 10 and 200 ppm, respectively). The registrant is requested to comment on/explain the 295 value.]

The statistically significant ($p<0.05$) increase in mean alkaline phosphatase levels in the 0.5 and 10 ppm male groups (not at 200 ppm) is likely due to two rats in each group which had values of 112 -131 IU/l; whereas, the next highest value in any group was 95. It is therefore not considered that the elevated levels were caused by Pyrinex administration. Aspartate transaminase was reduced in all male groups ($p<0.001$) and in the 10 ppm female group ($p<0.01$). An adverse effect of compound administration usually results in an increase in the level of this enzyme, and it is therefore felt that these observations are not of toxicological significance.

Gamma-glutamyl transpeptidase (G-GTP) levels were higher than controls ($p<0.001$) in the 200 ppm males and lower in all three female groups ($p<0.01$) The mean value table on page 41 of the report shows the male 0.5 ppm group mean to be 1.3 IU/l. As indicated in Table 5 of this review, the mean, based on individual values, is 0.9 IU/l. This reviewer does not feel that any of these differences are of toxicological significance and therefore will not request the Registrant to clarify the difference between mean and individual values. [Possibly a typographical error as the mean control value was 1.3 IU/l.]

There was a statistically significant ($p<0.01$ or 0.001) increase in the mean fasting blood sugar values in all dosed male groups. No apparent difference was observed in females. Mean urea values were increased in all male groups ($p<0.01$ at 0.5 and 10 ppm, not significant at 200 ppm) amd decreased in all female groups ($p<0.01$, 0.001 and 0.05 at 0.5, 10 and 200 ppm, respectively). Mid-dose males and females as well as high-dose males had mean bilirubin values above respective controls ($p<0.05$).

Regarding plasma proteins, there were statistically significant decreases in the mean group values for the following: total protein - 200 ppm males ($p<0.001$); alpha 1 globulin - 0.5 ppm females ($p<0.001$); and beta globulin - 10 and 200 ppm males ($p<0.05$ and 0.001, respectively). Concerning electrolytes, there were statistically significant increases in potassium (200 ppm male $p<0.05$) and phosphorus (200 ppm male and 10 ppm female $p<0.01$) with decreases in chloride (10 ppm male $p<0.05$) and calcium (10 and 200 ppm males $p<0.01$).

Urinalysis

As noted in Table 6, high-dose males (200 ppm) had statistically significant lower mean values than controls for volume ($p<0.05$) and pH ($p<0.001$) along with a higher value for specific gravity ($p<0.05$). In females, the only statisically significant difference from control was an increase in pH at 10 ppm ($p<0.05$). In addition, there were 7 high-dose males vs 2 controls which had grade "2" protein (no apparent difference in females).

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Table 6

SELECTED URINALYSIS FINDINGS FOR RATS ADMINISTERED PYRINEX (CHLORPYRIFOS) FOR 13 WEEKS

Dose ppm	0	0.5	10	200
MALES (10/group)				
Volume (ml)	8.3[a]	7.5	7.7	5.9*
	2.4[b]	2.1	1.7	2.6
pH	7.00	7.01	6.99	6.49**
	0.24	0.38	0.20	0.14
Specific Gravity	1.041	1.042	1.044	1.052*
	0.007	0.013	0.009	0.014
Protein (Grading)		(Incidence)		
0	3	0	3	0
1	5	8	6	3
2	2	2	1	7
FEMALES (10/group)				
Volume (ml)	4.4	5.4	5.4	4.1
	1.0	1.7	1.5	1.1
pH	6.20	6.36	6.65*	6.14
	0.24	0.32	0.40	0.28
Specific Gravity	1.047	1.046	1.042	1.046
	0.005	0.005	0.006	0.009
Protein (Grading)		(Incidence)		
0	0	3	3	2
1	10	7	7	7
2	0	0	0	1

a = Mean
b = Standard Deviation
* = Statistical significance from control $p<0.05$.
** = Statistical significance from control $p<0.01$.

Data extracted from Table 8, pages 44-46 of the report.

Ophthalmoscopy

There did not appear to be any treatment related changes. Hyperreflective areas were noted in one rat from each of the following groups: male - control and 200 ppm; female - 200 ppm. One control female was reported to have an opaque lens.

Organ Weights

Absolute (g) and relative (percent of body weight) mean liver weights of the 200 ppm female group were heavier ($p<0.01$) than the control value (Table 7). The 0.5 ppm female livers were also heavier than the control (absolute, $p < 0.05$; relative, not significant). There was no apparent difference in the liver weight of males.

The mean group adrenal weight for the 200 ppm females was heavier than control (absolute, $p<0.05$; relative, not significant). Omission of two pair of adrenals in the 200 ppm group reported as "abnormal at necropsy" results in an absolute mean of 74.0 mg (instead of 76.8 mg) compared with a control value of 68.8 mg. This difference of 5.2 mg is less than one-half of one control Standard Deviation (11.0). No apparent differences between treated and control adrenal weights were noted in males.

Mean absolute male kidney weight of the 200 ppm group was less than control ($p<0.05$). The difference was less than one Standard Deviation. No difference in mean adrenal weights was observed in females.

[Page 28 of the report states Organ Weight Analysis Tables 10 and 11, Appendices 14 and 15. These should be Appendices 13 and 14.]

Macroscopic Findings

There were no unusual gross obsservations at necropsy which appeared to distinguish treated from control animals.

Microscopic Findings

No microscopic changes were regarded as related to treatment. Observed findings were: renal basophilic tubules and hydronephrosis; uterine hydrometra; duodenal focal epithelial metaplasia; pulmonary accumulation of macrophages; adrenal cortical focal hyperplasia; testicular degeneration of germinal epithelium; and prostatitis.

Table 7

MEAN ORGAN WEIGHTS FOR RATS RECEIVING PYRINEX BY DIETARY ADMIX FOR 13 WEEKS

	Males				Females			
ppm	0	0.5	10	200	0	0.5	10	200
ABSOLUTE (g)								
Body Weight (g)	489.8	498.7	485.9	482.1	252.2	260.1	255.6	262.4
Brain	2.06	2.05	2.06	2.06	1.89	1.89	1.91	1.90
Liver	20.21	20.55	19.41	19.38	9.65	10.30*	9.89	10.61**
Spleen	0.824	0.822	0.832	0.809†	0.490	0.498	0.485†	0.479
Kidneys ††	3.73	3.70	3.52	3.50*	2.05	2.13	2.15	2.15
Adrenals ††	60.6	55.5	59.1†	57.4†	68.8	67.0	65.4	76.8*
Testes ††	3.538	3.450	3.573	3.407	--	--	--	--
RELATIVE (percent of body weight)								
Brain	0.42	0.41	0.43	0.43	0.75	0.73	0.75	0.73
Liver	4.11	4.12	3.98	4.02	3.82	3.97	3.87	4.05**
Spleen	0.17	0.16	0.17	0.17†	0.19	0.19	0.19†	0.18
Kidneys ††	0.76	0.74	0.73	0.73	0.82	0.82	0.84	0.82
Adrenals †† (x 1000)	12.36	11.13	12.14†	11.97†	27.41	25.87	25.66	29.33
Testes †† (x 1000)	0.73	0.69	0.74	0.71	--	--	--	--

* = Significantly different from control, $p < 0.05$
** = Significantly different from control, $p < 0.01$
† = 19 rats
†† = Paired organs; values is sum of each weighed individually.

NOTE: All groups 20 rats/sex (unless otherwise noted)

Data extracted from Tables 10 and 11, pages 48 and 49 of the report.

IV. Discussion

Homogeneity data were considered to be only within questionably acceptable limits at Week 1 (ranges were 1263-1790, 172-247 and 9.9-12.4 ppm at 1500, 200 and 10 ppm). Week 3 values were acceptable with ranges of 1418-1471, 179-197 and 9.0-10.5 ppm. Stability data were included in the report and were considered to be acceptable.

Pyrinex at 200 ppm in the diet resulted in a significant decrease in body weight gain during approximately the first half of the study in males and during the first three weeks in females. By week 13, all treated groups had mean weight values about the same as controls. An increase in food consumption in the 200 ppm males and females was noted during about the time of increase in body weight gain toward control levels. [The authors of the study stated, "On the basis of the present work it is not possible to fully explain the cause of the increased food consumption in the high dosage group, but it would be reasonable to assume that it is connected with the reduced acetyl cholinesterase activity leading to increased muscular action." The reviewer questions this statement and suggests the possibility that increased food consumption goes along with the body weight gain observed. It is not felt that the data noted regarding this parameter is of serious consequence in assessing the toxicity of the chemical.]

Packed cell volume (PCV), hemoglobin (HB) and erythrocyte (RBC) group means were significantly ($p<0.05$ or 0.01) below control values in males and females administered 200 ppm. Even though these reduced values were within what would be considered a "normal" range, the fact that all three parameters were below control in both sexes plus corroboration by the individual animal data, there appears to be the possibility that these high-dose rats may have bordered on anemia. A study of longer duration might shed light as to whether this apparent effect is an artifact, a true finding or a transient result of Pyrinex administration. In addition, destruction of erythrocytes is also suggested by an elevation of plasma bilirubin values in the 10 and 200 ppm male and female groups.

There was a statistically significant ($p<0.01$ or 0.001) dose response increase in cholinesterase inhibition observed in all Pyrinex male groups. In females, a significant ($p<0.001$) increase was noted at 200 ppm. The value reported for the 10 ppm group mean appears to be unusual (control = 3300; 0.5 ppm = 3639; 10 ppm = 295; and 200 ppm = 1427) and the Registrant is requested to clarify this value before the study is considered acceptable.

It is felt that the significant ($p<0.05$) increase in alkaline phosphatase levels in the 0.5 and 10 ppm male groups is due to two rats with high values in each group, and not to the test article (no elevation was observed in high-dose males nor in any female groups). Aspartate transaminase levels in all Pyrinex male groups and the 10 ppm female group were significantly ($p<0.01$) below respective controls. These lower values are not consideered to be of toxicological importance as an adverse effect is usually associated with an increase.

Gamma-glutamyl transpeptidase (G-GTP) levels were higher than control in the 200 ppm male group ($p<0.001$) and lower in females (all treated groups, $p<0.01$). It is not considered that the elevation/depression is of toxicological significance. Refer to footnote "d" on Table 5 for a discrepancy between individual and mean values. An increase ($p<0.01$ or 0.001) in glucose in all male dose groups with no apparent difference in the females, is not considered to be of toxicological significance. The 0.5 and 10 ppm male groups had elevated urea values; whereas, in females, all three groups had values below control ($p<0.05$-0.001). Bilirubin elevation (note reference under "hematology" discussion) was observed in 10 and 200 ppm males ($p<0.05$) and in the same female groups (10 ppm $p<0.05$; 200 ppm not sig.). The total protein level was below control in the 200 ppm male group. Changes (though statistically significant in some instances) which were observed in the above parameters do not appear to be a test article induced toxicologic finding as not only were the differences relatively small, but in most cases, occurred in only one sex. In addition, there was no histopathology which appeared to substantiate possible tissue damage. There were isolated differences in electrolyte values, but these did not appear to have a pattern related to Pyrinex administration.

Urine volume, in the male high dose only, appeared to be less ($p<0.05$) than control. In addition, this group also had a lower mean pH ($p<0.01$) and a higher specific gravity. These differences were not observed in females. A protein grading of "2" was noted in 7 high-dose males (1 or 2 in control or other dose groups) and only 1 high-dose female (0 in the three other groups). The decreased volume and pH as well as a larger number of males having a higher protein grading appear to be the result of Pyrinex treatment.

Absolute and relative mean liver weights were greater than the control value for the 200 ppm group females, but not for males. Because of the relatively small increase in the female weights, the apparent lack of an increase in males and no reported adverse hepatic histopathology, it is felt that the observed increases are not of toxicological significance. The male kidney and female adrenal weights which differed from control appear to be a normal biological variation and are not considered to be related to Pyrinex administration.

Neither macroscopic nor microscopic findings indicated any treatment related changes.

V. Conclusion

The Cholinesterase No Observed Effect Level (NOEL) was not attained in males (increased inhibition at 0.5 ppm - LDT) and was 0.5 ppm in females. The Systemic Toxicity NOEL was 10 ppm and the Systemic Toxicity Lowest Observed Effect Level (LOEL) was 200 ppm based on a decrease in body weight gain and possible anemia.

VI. Recommendation

Classification: Supplementary

This study may be upgraded depending upon the Registrant's response regarding the female cholinesterase values.

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Pages 64 through 77 are not included.

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006851

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 8/22/88
Section V, Tox Branch (TS-769C)
Secondary reviewer: Kerry Dearfield, Ph.D. Kerry Dearfield 8.23.88
Section SMSS, Tox Branch (TS-769C)
Date: August 22, 1988

DATA EVALUATION REPORT

CHEMICAL: PYRINEX (Chlorpyrifos) Tox. Chem. No.: 219AA

EPA File Symbol: 11678-UL

STUDY TYPE: In Vitro Chromosomal Aberration Assay

ACCESSION NUMBER: 404364-09

SYNONYMS/CAS No.: 2921-88-2; CAS # 2921-88-2

SPONSOR: Makhteshim-Agan (America), Inc.

TESTING FACILITY: Arthur D. Little, Inc.

TITLE OF REPORT: In Vitro Chromosomal Aberration Assay on Pyrinex (Chlorpyrifos)

AUTHOR(S): K. S. Loveday, Ph.D.

STUDY NUMBER (S): 59487-CAA

REPORT ISSUED: October 2, 1987

CONCLUSION(S) - Executive Summary:

Pyrinex (Chlorpyrifos) was tested in an in vitro chromosomal aberration assay with and without S-9 activation. Concentrations assayed were: non-activation - 10 hour assay = 1.56, 3.12, 5.2, 10.4, 15.6, 31.2, 52, 104 & 156 ug/ml; 19-20 hour assay = 0.975, 1.47, 2.93, 4.89, 9.75, 14.7, 29.3, 48.9, 97.5 & 147 ug/ml --- activation - two 10 hour assays = 1, 1.5, 3, 5, 10, 15, 30, 50 & 100 ug/ml and 2.95, 4.95, 9.85, 14.8, 29.6, 49.4, 98.5 & 296 ug/ml; 19-20 hour assay = 9.75, 14.7, 29.3, 48.9, 97.5, 147 & 293 ug/ml. Positive controls were Mitomycin C (non-activation) and cyclophosphamide (activation). Cytotoxicity was shown in both non-activated as well as in activated assays. Pyrinex did not appear to cause chromosomal aberrations. Positive controls caused appropriate mutagenic response.

Classification: Acceptable

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A. MATERIALS

1. Test Material: Name: PYRINEX (Chlorpyrifos)
Description: solid; stable at room temperature

Batch #: 489205 Purity: 96.8%
Contaminants: None reported
Solvent used: Dimethylsulfoxide (DMSO); Fisher Scientific; Lot Nos. 865895, 853290 and 862264
Other comments: None

2. Control Materials:

Negative: Solvent (DMSO)
Solvent/final concentration: 0.5%
Positive: Non-activation - Mitomycin C, Sigma Chemical Co., Lot No. 123F-0463; 0.3 or 5.0 ug/ml
Activation - Cyclophosphamide, Sigma Chemical Co., Lot No. 33F-0157; 50 ug/ml

3. Activation: S9 derived from Aroclor 1254, induced, rat, liver

S9 Composition: Source was Microbiological Associated, Lot/Batch 296

4. Test compound concentrations used:

Non-Activated conditions:
19-20 hour assay: 0.975, 1.47, 2.93, 4.89, 9.75, 14.7, 29.3, 48.9, 97.5 and 147 ug/ml
10 hour assay: 1.56, 3.12, 5.2, 10.4, 15.6, 31.2, 52, 104 and 156 ug/ml

Activated conditions:
19-20 hour assay: 9.75, 14.7, 29.3, 48.9, 97.5, 147 and 293 ug/ml
Two 10 hour assays:
1, 1.5, 3, 5, 10, 15, 30, 50, and 100 ug/ml
2.95, 4.95, 9.85, 14.8, 29.6, 49.4, 98.5 and 296 ug/ml

5. Test Cells: CHO cells used in the assay were obtained from Dr. Sheila Galloway at Litton Bionetics, Kensington, MD in 1983.

Properly maintained: Yes

Cell line or strain periodically checked for Mycoplasma contamination? Yes

Cell line or strain periodically checked for karyotype stability? Not stated

B. TEST PERFORMANCE

1. Cell treatment:

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a. Cells exposed to test compound for: 19-20 or 10 hours (non-activated); 2 hours (activated)

b. Cells exposed to positive controls for: 19-20 or 10 hours non-activated); 2 hours (activated)

c. Cells exposed to negative and/or solvent controls for: 19-20 or 10 hours (non-activated); 2 hours (activated)

2. Protocol:

A copy of the Materials and Experimental Design sections of the report are appended.

3. Preliminary cytotoxicity assay: None mentioned in the report.

4. Cytogenetics assay: Tables 1-10 are photocopies of Tables 1-10, pages 20-29 of the report.

In the two non-activated assays, Pyrinex was toxic as evidenced by the lack of cells in the flasks: 104 ug/ml, 10 hour assay; 97.5 ug/ml, 19-20 hour assay (Tables 1 and 2). There was also a suggestion of slight toxicity in the 52.0 ug/ml group in the 10 hour assay (duplicate samples) mitotic index for: DMSO = 5.4 & 6.0; positive control = 1.6 & 1.6; 5.2 ug/ml = 6.2 & 5.4; 15.6 ug/ml = 8.4 & 6.8; 52.0 ug/ml = 4.6 & 3.2.

There were no apparent increases in percent of cells with chromosomal aberrations in either the 10 or 19-20 hour non-activated assays when gaps were excluded (Tables 6 and 7). When gaps were included, the 52.0 ug/ml concentration (highest concentration with cells observed in 10 hour assay), had 36 & 31% of the cells with aberrations compared with 15% & 10% for DMSO solvent and 57% & 55% with Mitomycin C (positive control). No apparent increases were observed in the 19-20 hour assay.

There were two 10 hour and one 19-20 hour experiments in the activation assays (Tables 3, 4 and 5)[cells exposed for 2 hours, incubated for remaining time in medium]. Toxicity was observed as follows (cells in flasks): 10 hour assay experiment 1 = no cells observed at 15 ug/ml, slightly reduced at 10 ug/ml; 10 hour assay experiment 2 = no cells observed at 14.8 ug/ml, slightly reduced at 9.85 ug/ml; 19-20 hour assay = no cells observed at 147 ug/ml, slightly reduced at 97.5 ug/ml.

In the activated assays, the author stated that there was a statistically greater (Table 8 does not indicate the statistical significance) percent of aberrant cells (with gaps excluded) in the 3 ug/ml group (11 & 12%) compared with values from the solvent (3 & 3%), 1 ug/ml (3 & 9%) or 10 ug/ml (4 & 7%) groups. This was not observed in experiment 2 (Table 9) where all values were similar. When gaps were included a statistically significant increase in cells with aberrations was noted at the 3 and 10 ug/ml concentrations (Table 8). There is the possible suggestion that an increase in percent of cells with aberrations (gaps included) also occurred in the 1 ug/ml group (5 & 17%) vs DMSO values of 3 & 4%). In the 19-20 hour activated assay, there were no apparent increases in the percent of cells with aberrations either excluding or including gaps. Cyclophosphamide positive controls increased the percent of cells with aberrations at least 4x the DMSO negative control mean values.

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5. Reviewer's discussion/conclusions:

Cytotoxicity was shown in both non-activated as well as in activated assays. In the non-activated assays with 10 or 19-20 hours exposure to the test chemical, there were no apparent increases in cells with aberrations compared with negative control (DMSO solvent) values. Of the two 10 hour activated assays performed, the only suggestion of an increase in the percent of cells with aberrations occurred at the 3 ug/ml concentration (mid-concentration) in one assay and was not observed in either this approximate concentration in the second assay or at the higher concentrations in either of the two assays. Therefore, it is not felt that Pyrinex in these activated assays causes a positive effect on chromosomal aberrations. The positive controls in the non-activated and activated assays caused appropriate mutagenic responses.

6. Was test performed under GLPs: Yes, Quality Assurance statement is present.

7. CBI appendix attached: None

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Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 8/22/88
Section V, Tox Branch (TS-769C)
Secondary reviewer: Kerry Dearfield, Ph.D. Kerry Dearfield 8-23-88
Section SMSS, Tox Branch (TS-769C)
Date: August 22, 1988

DATA EVALUATION REPORT

CHEMICAL: PYRINEX (Chlorpyrifos) Tox. Chem. No.: 219AA

EPA File Symbol: 11678-UL

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION NUMBER: 404364-11

SYNONYMS/CAS No.: 2921-88-2; CAS # 2921-88-2

SPONSOR: Makhteshim-Agan (America), Inc.

TESTING FACILITY: Arthur D. Little, Inc.

TITLE OF REPORT: Evaluation of Pyrinex in the Ames Mutagenesis Assay

AUTHOR(S): K. S. Loveday, Ph.D., K. M. B. Findlen, B.S., MT (ASCP) and S. Yadlon, B.S.

STUDY NUMBER(S): 59487-AMA

REPORT ISSUED: August 7, 1987

CONCLUSION(S) - Executive Summary:

Pyrinex (Chlorpyrifos) was tested in Salmonella strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 (in the presence and absence of S-9) at concentrations of 30, 100, 300, 1000, 3000 and 10000 ug/plate. DMSO was the solvent and negative control; positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-anthramine. Pyrinex was not toxic nor did it appear to increase over control values the number of revertant colonies/plate. Positive controls caused appropriate mutagenic responses.

Classification: Acceptable

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A. MATERIALS

1. Test Material: Name: PYRINEX (Chlorpyrifos)
Description: off white crystals, stable at room temperature
Batch #: 489205 Purity: 96.8%
Contaminants: None reported
Solvent used: Dimethylsulfoxide (DMSO); Fisher Scientific; Lot No. 853290
Other comments: None

2. Control Materials:

Negative: Solvent (DMSO)
Solvent/final concentration: DMSO
Positive: Non-activation - Sodium azide 10 ug/plate TA 100, TA 1535
2-Nitrofluorene 10 ug/plate TA 98, TA 1538
9-Aminoacridine 50 ug/plate TA 1537
Other: None

Activation - 2-Aminoanthracene (2-anthramine) 5 and 10 ug/plate
Other: None

3. Activation: S-9 derived from Aroclor 1254, induced, rat, liver

S-9 mix composition: Source - Microbiological Associates
Batch No. - R338
Date Prepared - 10/29/86

4. Test organisms: S. typhimurium strains - TA 98, TA 100, TA 1535, TA 1537 and TA 1538

Properly maintained: Yes

5. Test compound concentrations used:

Non-activated: ug/plate = 30, 100, 300, 1000, 3000 and 10000
Activated: ug/plate = 30, 100, 300, 1000, 3000 and 10000

B. TEST PERFORMANCE

1. Type of Salmonella assay: standard plate test

a. Protocol: A copy of the Materials and Experimental Design sections of the report are appended.

2. Preliminary cytotoxicity assay: None mentioned in the report.

3. Mutagenicity assay:

Table 1 (a photocopy of Table 1, page 14 of the report) presents mean/summary data for negative controls (DMSO with and without S-9), positive controls (sodium azide, 9-aminoacridine and 2-nitrofluorene without S-9; 2-anthramine with S-9) and the following ug/plate of Pyrinex with and without S-9: 30, 100, 300, 1000, 3000 and 10000.

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The DMSO and Pyrinex were tested against 5 strains of Salmonella; whereas, the positive controls were tested against appropriate strains. Three replicates in each assay were performed. Pyrinex was not toxic nor did it precipitate at 10000 ug/plate. Pyrinex was negative (non-mutagenic) in all 5 strains with and without S-9. The study authors stated that the average DMSO background counts were within historical ranges. Positive controls caused appropriate mutagenic responses. Two concentrations of 2-anthramine were used to ensure an adequate positive response in all strains.

4. Reviewer's discussion/conclusions:

All Pyrinex concentrations resulted in mean total revertant colonies/plate approximately the same as DMSO negative control values. Positive controls had mean spontaneous revertants/plate at least ten times DMSO or Pyrinex values in most instances (none less than three times control). As Pyrinex did not appear to increase the number of revertant colonies/plate over controls, in the presence or absence of a metabolic activation system, the test article is not considered to be mutagenic in this assay.

This Salmonella Mutagenicity assay is therefore considered acceptable.

5. The test was performed under GLPs and a Quality Assurance statement was present.

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Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 8/23/88 006851
Section V, Tox Branch (TS-769C)
Secondary reviewer: Kerry Dearfield, Ph.D. Kerry Dearfield 8.23.88
Section SMSS, Tox Branch (TS-769C)
Date: August 22, 1988

DATA EVALUATION REPORT

CHEMICAL: PYRINEX (Chlorpyrifos) Tox. Chem. No.: 219AA

EPA File Symbol: 11678-UL

STUDY TYPE: Mammalian cells in culture gene mutation assay in CHO/HGPRT cells

ACCESSION NUMBER: 404364-10

SYNONYMS/CAS No.: 2921-88-2; CAS # 2921-88-2

SPONSOR: Makhteshim-Agan (America), Inc.

TESTING FACILITY: Arthur D. Little, Inc.

TITLE OF REPORT: CHO/HGPRT In Vitro Mammalian Cell Mutation Assay on Pyrinex (Chlorpyrifos)

AUTHOR(S): A. S. Tu, Ph.D.

STUDY NUMBER(S): 59487-CMA

REPORT ISSUED: August 4, 1987

CONCLUSION(S) - Executive Summary:

Pyrinex (Chlorpyrifos) was tested in the mammalian cell CHO/HGPRT gene mutation assay at concentrations of 5-75 ug/ml in the non-activation study and 30-1000 ug/ml in the activation study. Cytotoxicity assays were performed in both the non-activation and activation studies (1.5-3748 ug/ml and 1.5-5000 ug/ml, respectively). DMSO (solvent) and the medium were negative control groups. Positive controls were ethylmethanesulfonate (non-activated) and dimethylnitrosamine (activated). Pyrinex showed cytotoxicity only in the non-activated study (at 150 ug/ml). "Pitting" of the plastic culture vessels occurred at 50 or 150 ug/ml (non-activated or activated, respectively). It appeared that the assay tested concentrations that were at the limits of solubility. There was no evidence that Pyrinex caused mutation in either the non-activated or activated studies. Positive controls caused appropriate mutagenic responses.

Classification: Acceptable

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A. MATERIALS

1. Test Material: Name - PYRINEX (Chlorpyrifos)
Description: off-white granular crystals; stable indefinitely at room temperature
Batch #: 489205 Purity: 96.8%
Contaminants: None reported
Solvent: Dimethylsulfoxide (DMSO); Fisher Scientific; Lot No. 853290
Other comments: None

2. Control Materials:

Negative: DMSO
Solvent/final concentration: DMSO
Positive: Non-activation - Ethylmethanesulfonate (EMS); 6.2 mg/ml in phosphate buffered saline (PBS). Concentration used = 248 ug/ml.

Activation - Dimethylnitrosamine (DMN); 10 mg/ml in phosphate buffered saline (PBS). Concentration used = 500 and 1000 ug/ml.

Further dilutions: if necessary, in PBS

3. Activation: S-9 derived from Aroclor 1254, induced, rat, liver

S-9 mix: Source - Litron Laboratories, Ltd.
Lot No. - F07.22
Storage conditions - -80°C.

4. Test Cells: Chinese hamster ovary (CHO) cells

Properly maintained: Not stated
Periodically checked for Mycoplasma contamination: Not stated
Periodically checked for karyotype stability: Not stated
Periodically "cleansed" against high spontaneous background: Not stated

5. Locus Examined: hgprt locus

6. Test compound concentrations used:

Non-activated: Range finding (ug/ml) = 1.5, 5, 15, 50, 150, 500, 1465 and 3748
Exp. 1 (ug/ml) = 5, 10, 25, 50 and 75
Exp. 2 (ug/ml) = 5, 10, 20, 30, 40 and 50

Activated: Range finding (ug/ml) = 1.5, 5, 15, 50, 150, 500, 1500 and 5000.
Exp. 1 (ug/ml) = 30, 50, 100, 300 and 1000
Exp. 2 (ug/ml) = 30, 50, 100, 300 and 1000

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound for: 16 hours (non-activated) and 5 hours (activated).
b. Cells exposed to positive controls for: 16 hours (non-activated) and 5 hours (activated)
c. Cells exposed to negative and/or solvent controls for: 16 hours (non-activated) and 5 hours (activated)
d. After washing, cells cultured for 7-9 days before cell selection
e. After expression, cells cultured for 7 days in selection medium to determine numbers of mutants and for 7 days without selection medium to determine cloning efficiency.

2. Protocol

A copy of the Materials and Experimental Design sections of the report are appended.

3. Preliminary cytotoxicity assay

CHO cells were seeded at 200 cells/culture flask (F12 medium with 10% FCS and 0.02M HEPES buffer pH 7.4). Test material concentrations were 1.5, 5, 15, 50, 500, 1465 and 3748 ug/ml in the non-activated system and 1.5, 5, 15, 50, 150, 500, 1500 and 5000 ug/ml in the activated system. Exposure was for 16 hours in the non-activated and 5 hours in the activated assays. After 6 days of incubation the cells were fixed, stained and the colonies counted. Toxicity was expressed as the relative surviving fraction (average colonies of treated cells divided by average colonies of the medium control).

Pyrinex was soluble in DMSO. The sample precipitated at 50 ug/ml and higher ("pitting" of the plastic flasks was noted in the non-activated assay). In the activated assay, "pitting" was noted at 150 ug/ml and higher.

The results are presented in Table 1 (without activation) and Table 2 (with activation). [These tables are photocopies of Tables 1 and 2 on pages 20 and 21 of the report.] There were no colonies present above 50 ug/ml in the non-activated assay. In the activated assay, colonies were present at 5000 ug/ml (highest dose tested). Based upon these results, the highest concentration of test material used in the mutation assays were 75 ug/ml (1st experiment) and 50 ug/ml (2nd experiment) in the non-activated assay and 1000 ug/ml in the activated assay.

4. Mutagenicity assay:

Tables 3 and 4 (photocopies of Tables 3 and 4, pages 22 and 23 of the report) present the results of experiments 1 and 2 of the non-activation assay. Cytotoxicity was observed at 50 and 75 ug/ml in exp. 1, but when 50 ug/ml was tested in exp. 2, no cytotoxicity was noted. The total number of mutant colonies as well as mutation frequency values of Pyrinex groups were equal to or less than the values observed with either medium control or DMSO vehicle. The EMS positive controls showed a much greater number of total mutant colonies

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(941, exp. 1; 537 exp. 2) and mutation frequency (1115.83, exp. 1; 422.93, exp. 2) than was noted for any other group (DMSO control 14 mutant colonies; 9.80 mutation frequency).

In the S-9 activation system (Tables 5 and 6 - photocopies of Tables 5 and 6, pages 24 and 25 of the report), the degree of cytotoxicity was less after this 5-hour exposure than with the 16-hour exposure in the non-activated system. Cytotoxicity was observed at 300 ug/ml and higher. Pyrinex was not soluble at these test concentrations under the assay conditions. There were no consistent mutagenic responses with the test chemical. In exp. 2, at 300 ug/ml, there was a statistically significant increase in the mutation frequency compared with the medium or DMSO control values (10.21 vs 1.37 and 0.56 for controls). The results in exp. 1 at the 300 ug/ml dose showed Pyrinex values less than the medium control (DMSO control = 0.00). The DMN positive controls (500 or 1000 ug/ml) in the two experiments had total mutant colony counts of 135-160 with mutation frequencies of 163.33-260.35.

5. Reviewer's discussion/conclusions:

The reason for the discrepency between the two non-activated experiments regarding cytotoxicity at 50 ug/ml is not clear. The study authors indicated that this difference may have been due to the precipitation of the chemical at that concentration. It is not felt that the difference has a bearing on the value of the study. In the non-activation preliminary cytotoxic study, precipitation of the test chemical was observed above 50 ug/ml. Although this was not noted in the activation assay, "pitting" was seen. The choice of up to 75 ug/ml in the non-activation mutagenicity assay and up to 1000 ug/ml in the activation mutation assay seems to be appropriate.

There was no apparent evidence of mutagenicity in either the non-activation or activation assays at the concentrations tested. The apparent increase in mutation frequency (statistically significant) observed at 300 ug/ml in the activation assay exp. 2 is not considered to be a positive mutagenic effect. This increase was not seen in exp. 1 at this concentration nor at 1000 ug/ml in either experiment. It is therefore considered that the elevated values were within the limits of biological variation. The positive controls in both non-activation as well as activation assays increased the mutation frequency many fold over control data, therefore causing an appropriate mutagenic response.

6. The test was performed under GLPs. A Quality Assurance statement was present.

CHLORPYRIFOS

Page_____ is not included in this copy.

Pages 113 through 124 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.