(EXCERPT 005108)

11-14-85

EPA: 68-01-6561
DYNAMAC No. 029E(1-3)
November 13, 1985

DATA EVALUATION RECORD

GRENADE (Cyhalothrin)

Chronic Toxicity and Oncogenicity Feeding Study in Mice

STUDY IDENTIFICATION: Colley, J., Dawe, S., Heywood, R., Almond, R., Gibson, W. A., Gregson, R., and Gopinath, C. Cyhalothrin: potential tumorigenic and toxic effects in prolonged dietary administration to mice. (Unpublished study No. CTL/C/1260 CTL [study No. PMO 400] prepared by Huntingdon Research Centre, Cambridgeshire, England, for Imperial Chemical Industries, Cheshire, England; dated May 31, 1984.) Accession No. 073214-073216.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 11-14-85

1. CHEMICAL: Grenade, cyhalothrin (ICI 146,814: PP563).

2. TEST MATERIAL: Cyhalothrin, batch No. YOO 102/010/005, was described as a brown viscous liquid. Its purity was not specified.

3. STUDY/ACTION TYPE: Chronic toxicity and oncogenicity feeding study in mice.

4. STUDY IDENTIFICATION: Colley, J., Dawe, S., Heywood, R., Almond, R., Gibson, W. A., Gregson, R., and Gopinath, C. Cyhalothrin: potential tumorigenic and toxic effects in prolonged dietary administration to mice. (Unpublished study No. CTL/C/1260 [study No. PMO 400] prepared by Huntingdon Research Centre, Cambridgeshire, England, for Imperial Chemical Industries, Cheshire, England; dated May 31, 1984.) Accession No. 073214-073216.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: Nov. 14, 1985

Robert J. Weir, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Robert J. Weir
Date: Nov. 14, 1985

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Chronic Toxicity/Oncogenicity
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 11-14-85

Pamela Hurley, Ph.D.
EPA Reviewer

Signature: Pamela Hurley
Date: 4/23/86

Edwin Budd
EPA Section Head

Signature: John Budd
Date: 5/5/86

7. CONCLUSIONS:

A. Under the conditions of the study cyhalothrin was not oncogenic when fed to mice for 104 weeks at levels of 20, 100, or 500 ppm in the diet. There was a significant increase in mammary adenocarcinomas in females receiving 100 and 500 ppm compared to controls; however, the concurrent control incidence was unusually low and the increased incidence was therefore judged not to be of biological significance. A LOEL for systemic chronic toxicity, based on decreased weight gain in males during the first 13 weeks of the study, was 500 ppm, and the NOEL was set at 100 ppm. The only other toxic effect noted was an increase in the number of animals observed with piloerection and hunched posture at a dose level of 100 ppm in males and females; this was of minimal toxicologic importance.

B. The study is considered Core Minimum; it has not been adequately demonstrated that the highest dose tested was a maximum tolerated dose.

8. RECOMMENDATIONS:

It is recommended that the sponsor provide the rationale for dose selection so reviewers can be ensured that a maximum tolerated dose was used in the chronic oncogenicity study.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

See Appendix A for details.

A. Materials and Methods:

1. The test material, cyhalothrin, batch No. YOO 102/010/005, was described as a brown viscous liquid. The purity was not specified. The dosed feed was tested for homogeneity and dietary stability prior to the start of treatment. At 3-monthly intervals during the study, samples of the diets were analyzed for cyhalothrin concentration.

2. Four main groups of 52 CD-1 mice of each sex, including an untreated control group that received the diet only, were administered the test material in the diet at concentrations of 0, 20, 100, and 500 ppm for 104 weeks (termination of study). In addition, four satellite groups of 12 mice of

---

[1]Only sections appropriate to the DER are included.

each sex, fed the same diet concentrations, were maintained for laboratory investigation and terminated at week 52 of study (interim sacrifice).

3. Animals were observed daily for toxic signs; palpations for masses were also performed. After the first four weeks the observations for clinical reactions to treatment and the palpations for masses were only conducted once per week. Body weights and food consumptions were appropriately measured weekly and recorded throughout the study. Water consumption was monitored daily and was actually measured during week 48. All cages were checked daily for dead and moribund animals.

4. Blood for hematology and blood chemistry testing and pooled urine samples from each cage for urinalysis were collected from all mice in the satellite groups prior to the interim (week 52) sacrifice and from 12 male and 12 female animals from each main group at the terminal (week 104) sacrifice. The following hematology measurements were taken: packed cell volume (PCV), hemoglobin (Hb), red cell count (RBC), mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV), total white cell count (WBC Total), differential count and platelet count (Plts).

   The following blood biochemistry measurements were taken: plasma urea nitrogen (urea N), plasma glucose, plasma total protein, plasma albumen (Alb), plasma globulin (Glob), plasma alkaline phosphatase (AP), plasma glutamic-pyruvic transaminase (GOT) and plasma cholesterol (Chol).

   The following urinalysis measurements were taken: volume, pH, specific gravity, protein concentration, glucose, and ketones.

5. At the interim sacrifice and at termination of the study, all surviving mice in the satellite and main groups respectively were killed using CO_2; these animals and those that died or were sacrificed moribund were subjected to an extensive gross examination. Major organs and all gross lesions were examined microscopically, when feasible, from all animals on study. Major organs were also weighed; the organ weights from mice that died during the course of te study were taken under the discretion of the pathologist. Samples of the following tissues were preserved for microscopic examination: adrenals, bone, brain (medullary, crebellar and cortical sections), caecum, duodenum, eyes, gall bladder, Harderian gland, head (nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx and middle ear), heart, ileum, jejunum, kidneys, liver (from at least two lobes, multiple sections when possible metastasis), lungs (all lobes and mainstem bronchi, multiple sections when possible metastasis), lymph nodes (cervical and mesenteric, multiple sections as above), mammary gland, midcolon, esophagus, ovaries, pancreas, pituitary, prostate,

salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (at least two levels), spleen, sternum (for bone marrow), stomach (glandular and non-glandular), testes, thymus (where present), thyroid (with parathyroid), trachea, urinary bladder, uterus (plus cervix) and all abnormal tissues. In addition, three coronal sections through the head were examined in ten males and ten females from each group and in any other animal in which there was evidence of disease.

6. Statistical Analysis: Analysis of variance was used to assess the significance of intergroup differences, and intergroup comparisons were assessed using the Student's t test. Tumor incidence was analyzed following adjustment for intergroup differences in mortality patterns by log rank methods as described by Peto et al.[2]

12. REPORTED RESULTS:

Dietary Analysis: The concentration of cyhalothrin in the test diets was analyzed at 13-week intervals throughout the study. The mean concentrations (from duplicate analyses) of the test material in the diets at 20, 100, and 500 ppm were within 9 percent of the nominal values, with the exception of one result at week 52 (which was found to be 21.5 percent for the 20-ppm diet). Homogeneity was determined from duplicate samples randomly taken from the top, middle, and bottom of the blender. The mean concentration ranges were 19.3 to 19.6 ppm for the 20-ppm level and 477 to 492 ppm for the 500-ppm level. Test material was stable in diets stored at ambient temperature in the animal rooms for at least 6 weeks. The mean concentrations at weeks 0, 3, and 6 were, respectivey, 19.3, 19.9, and 19.2 ppm for the 20-ppm level and 487, 492, and 494 ppm for the 500-ppm level at the same sampling periods.

Clinical Observations and Mortality: There was an increased incidence of piloerection in the mice at the highest dose (500 ppm) tested, particularly in males. This observation was also noted in the male mice in the mid-dose (100 ppm) group (Table 1). There was also a higher incidence of hunched posture in the highest dose groups compared to the control groups. This increased incidence continued throughout most of the study (Table 1). In the final week of the study, the incidences of both findings among treated and control mice were considered by the authors to be age-related rather than treatment-related changes.

---

[2] WHO International Agency for Research on Cancer (1980). Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal. Supplement 2, pp. 311-426.

TABLE 1. Summary of Clinical Observations at Selected Intervals in Mice Fed Cyhalothrin

Finding/Dose Group (ppm)	Percentage[a] of Animals with Finding at Week					
	4	13	26	52	78	104
				Males		
Piloerection						
Control	0	6	18	32	45	52
20	0	3	19	27	46	84
100	2	19	36	46	47	73
500	78	78	73	81	87	95
				Females		
Control	0	0	3	5	10	19
20	3	0	7	18	21	48
100	6	3	8	8	5	44
500	38	34	22	25	38	50
Hunched Posture						
				Males		
Control	2	0	0	0	0	39
20	0	2	0	0	5	26
100	0	2	3	2	5	47
500	6	19	20	30	18	32
				Females		
Control	0	0	0	0	2	19
20	3	0	0	0	0	5
100	0	3	2	3	3	24
500	8	6	3	7	9	9

[a] $\frac{\text{Number of mice showing finding during week}}{\text{Number of mice surviving at start of week}} \times 100$

Mortality was similar among all groups with the exception of a slightly increased mortality at 104 weeks in males receiving 100 ppm. Survival at study termination ranged from 27-40 percent in male groups and 38-48 percent in female groups (Table 2).

Body Weights: The mean weight gain in males receiving 500 ppm was significantly lower than in the control males during the first 13 weeks of the study, which resulted in an overall decreased weight gain for the 104 weeks of the study (Table 3). The mean body weight of the males receiving 500 ppm was 10 percent lower than controls at week 13 but only 2 percent lower at week 104. Mean body weights of females receiving 20 ppm were higher than controls throughout most of the study; they gained more weight than controls during the first 26 weeks.

Food Consumption: Mean food intake was slightly higher in the male dosed groups throughout the study when compared to controls, with a statistically significant increase in the high-dose group. A significant increase in food consumption was also reported for female mice in the low-dose (20 ppm) group when compared to controls during the first 26 weeks; however, over the 104 weeks of the study the difference from the controls was not significant (Table 4).

Hematology: Hematological values were similar but with some sporadic variability for dosed and control mice; however, these differences were not considered to be of toxicological significance.

Biochemistry: At week 100, there were significant ($p < 0.05$ except for 500-ppm males, which was $p < 0.01$) increases in mean values of serum glutamic oxaloacetic transaminase (SGOT) for both male and female mice receiving 100 and 500 ppm and significant increases in mean values of serum glutamic pyruvic transaminase (SGPT) for female mice in all dosed groups (Table 5). These increases in mean enzyme levels were due to some abnormally high individual levels and were considered to be age-related rather than compound-related changes.

There were minor differences noted in glucose, globulin, and urea nitrogen; however, these differences were not consistent with time or dose and were not considered to be of toxicological significance by the report authors.

Urinalysis: Urinalysis parameters were similar in control and dosed groups.

Organ Weights: At study termination, the mean ovarian weights of female mice receiving cyhalothrin were significantly lower (0.068-0.107 g) when compared to the controls (0.274 g). This decrease was associated with a decreased incidence of distension of the periovarian sacs noted in dosed females. All other mean organ weights were similar among treated and control mice. A slight but significant

TABLE 2. Mortality and Percent Survival of Mice Fed Cyhalothrin for 104 Weeks

Dose Group[a] (ppm)	Mortality (Percent Survival) at Week				
	13	26	52	78	104
Males					
Control	2(96)	3(94)	5(90)	10(81)	31(40)
20	0(100)	1(98)	5(90)	15(71)	34(35)
100	0(100)	1(98)	5(90)	16(69)	38(27)
500	1(98)	4(92)	8(84)	15(71)	35(33)
Females					
Control	0(100)	1(98)	4(92)	11(79)	27(48)
20	2(96)	3(94)	9(83)	14(73)	32(38)
100	0(100)	1(98)	5(90)	13(75)	27(48)
500	0(100)	1(98)	4(92)	9(83)	32(38)

[a]Fifty-two mice per group per sex (main group).

TABLE 3. Mean Body Weight Gain of Mice Fed Cyhalothrin for 104 Weeks

Dose Group (ppm)	Mean Weight Gain in the Intervals Between Weeks				
	0-13	13-26	26-52	52-104	0-104
Males					
Control	11.4 ± 3.54	1.1 ± 2.82	3.7 ± 2.50	2.1 ± 4.13	18.0 ± 3.97
20	12.2 ± 3.79	0.6 ± 3.12	4.8 ± 3.33*	2.0 ± 4.77	20.3 ± 7.14
100	10.7 ± 2.94	2.7 ± 2.52	4.1 ± 3.19	0.6 ± 5.18	19.3 ± 5.21
500	6.2 ± 3.90***	1.5 ± 3.82	3.0 ± 3.41	1.8 ± 2.58	13.9 ± 3.25*
Females					
Control	5.5 ± 2.59	1.9 ± 2.35	3.4 ± 2.87	3.5 ± 3.15	13.8 ± 5.28
20	7.3 ± 3.29***	3.0 ± 2.75*	4.3 ± 3.54	2.0 ± 4.24	15.6 ± 5.10
100	6.8 ± 3.01*	1.5 ± 2.80	3.9 ± 3.19	2.8 ± 4.18	14.7 ± 5.86
500	5.6 ± 2.78	2.1 ± 2.33	4.3 ± 3.00	2.5 ± 4.85	15.8 ± 5.04

*Statistically significantly different from control at $p < 0.05$.

***Statistically significantly different from control at $p < 0.001$.

TABLE 4. Mean Food Consumption of Mice Fed Cyhalothrin for 104 Weeks

Dose Group (ppm)	Mean Food Consumption (g/mouse/week) in the Intervals Between Weeks				
	0-13	14-26	27-52	53-104	1-104
Males					
Control	27 ± 2.1	26 ± 2.7	28 ± 3.7	27 ± 2.7	27 ± 2.2
20	28 ± 1.4*	28 ± 2.4	30 ± 3.1	30 ± 4.4	29 ± 3.1
100	28 ± 1.5**	29 ± 2.7**	30 ± 3.3	29 ± 1.8	28 ± 1.5
500	27 ± 1.5	29 ± 2.5*	32 ± 3.5**	30 ± 4.1	30 ± 3.1*
Females					
Control	24 ± 1.6	24 ± 1.6	25 ± 2.0	26 ± 2.5	25 ± 2.0
20	26 ± 2.0***	25 ± 1.6*	26 ± 1.9	27 ± 3.4	27 ± 2.4
100	24 ± 1.3	25 ± 2.0	26 ± 2.9	26 ± 2.6	26 ± 1.8
500	24 ± 1.8	24 ± 2.5	25 ± 2.5	25 ± 2.7	25 ± 2.2

*Statistically significantly different from control at $p < 0.05$.

**Statistically significantly different from control at $p < 0.01$.

***Statistically significantly different from control at $p < 0.001$.

TABLE 5. Serum Enzyme Levels (mU/mL) in Mice Fed Cyhalothrin for 104 Weeks

Dose Group ppm	SGOT		SGPT	
	Week 50[a]	Week 100[b]	Week 50[a]	Week 100[b]
Males				
Control	54 ± 6.4	52 ± 10.8	47 ± 10.3	51 ± 38.6
20	71 ± 36.7	61 ± 18.1	47 ± 18.1	62 ± 25.4
100	57 ± 21.9	80 ± 34.5*	45 ± 13.9	83 ± 56.4
500	71 ± 26.3	88 ± 66.7**	52 ± 27.7	80 ± 75.2
Females				
Control	67 ± 17.2	71 ± 22.6	47 ± 19.1	36 ± 11.7
20	84 ± 42.2	80 ± 29.6	50 ± 35.9	63 ± 51.7*
100	72 ± 24.0	118 ± 63.4*	47 ± 25.7	59 ± 34.6*
500	59 ± 8.7	100 ± 39.4*	40 ± 13.5	54 ± 16.2*

[a]Results from satellite groups.

[b]Results from main groups.

*Statistically significantly different from control at $p < 0.05$.

**Statistically significantly different from control at $p < 0.01$.

increase in mean brain weight was noted at the 12-month sacrifice in males receiving 500 ppm. However, this was not considered of biological importance because the brain weights were within the normal range and there were no brain weight changes at terminal sacrifice.

Gross Pathology: There were no gross findings in mice that were considered to be related to dosing. A slight increase in incidence of subcutaneous masses in females was noted (3/52 in control versus 7/52 and 6/52 in the 100- and 500-ppm groups, respectively); a marginal decrease in incidence of distension of the peri-ovarian sac (18/52 in controls and 16/52, 14/15, and 10/52 in the 20-, 100-, and 500-ppm groups of females, respectively) and an increase in incidence of thickening of the non-glandular epithelium of the forestomach (1/52 in controls and 10/52, 13/52, and 9/52 in the 20-, 100-, and 500-ppm groups of females, respectively) were noted. There were no corresponding histologic correlates.

Histopathology: Table 6 summarizes the incidence of neoplastic lesions. There was an increased incidence of mammary adenocarcinomas in female mice receiving cyhalothrin at 100 ppm ($p = 0.03$) or 500 ppm ($p = 0.04$). This was supported by a positive trend analysis ($p = 0.016$). However, there was a lack of a consistent dose-related response and the incidence was slightly higher than the laboratory's historical range (2-12%; average of 17 studies was 81/1156 or 7.0%); therefore, the increased incidence was not considered to be related to dosing. Occurrence of other tumors was incidental, small numbers were found but there were no dose-related increases.

Nonneoplastic lesions considered of toxicologic importance were not seen histologically. There was disseminated amyloidosis in several organs but no apparent increase in dosed groups; it was the most common factor contributing to death.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "the higher incidence of mammary tumors noted in females of some treated groups in comparison to the controls is not unduly at variance with the incidence normally seen in this strain of mouse at our laboratory. This finding is, in our opinion, not an indication of the carcinogenic potential of cyhalothrin." There were signs of minimal toxicity for male and female mice receiving 500 ppm cyhalothrin and male mice receiving 100 ppm. The authors considered the LOEL for chronic systemic toxicity to be 100 ppm and the NOEL to be 20 ppm.

B. A signed quality assurance statement, dated 22/3/84, was present.

TABLE 6. Neoplastic Lesions in Mice Fed Cyhalothrin for 104 Weeks[a]

Organ/Neoplasm	Males/Dose Level (ppm)				Female/Dose Level (ppm)			
	0	20	100	500	0	20	100	500
Lymphoreticular	(64)[b]	(64)	(64)	(64)	(64)	(64)	(64)	(64)
leukemias and lymphomas	2	6	7	2	9	10	8	14
Lung	(64)[b]	(63)	(64)	(64)	(63)	(64)	(64)	(64)
adenoma	7	5	4	7	6	6	0	7[c]
adenocarcinoma	10[c]	4	10	8	5[c]	8	6[d]	4
Liver	(64)[b]	(63)	(64)	(62)	(62)	(64)	(63)	(64)
benign	9	9	9	6	1	2	1	0
malignant	9	6	11	2	0	1	0	0
Harderian gland	(64)[b]	(62)	(64)	(63)	(62)	(64)	(63)	(64)
adenoma	5	4[b]	3	1	3	3	4	1
Mammary gland					(52)[b]	(52)	(52)	(52)
adenocarcinoma					1	0	7	6
Uterus					(63)[b]	(63)	(63)	(63)
leiomyoma					1	0	3	2
leiomyosarcoma					0	0	0	3
total tumors					1	0	3	5
Ovary								
granulosa cell tumor					(62)	(62)	(64)	(63)
					2	0	2	0

[a]If a tumor occurred only once in any group it was not included in this table.

[b]Number of tissues examined; includes 10-12 animals sacrificed at 12 months (except for mammary gland) since laboratory historical data did not include animals 12 months on study.

[c]One neoplasm occurred at the 12-month sacrifice.

[d]Two neoplasms occurred at the 12-month sacrifice.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The protocol was complete and adequate to assess the oncogenicity and chronic toxicity of cyhalothrin. The summary data presented in the report were supported by individual animal data and the summary data were accurate. The report was well organized and well written. Under the conditions of the study the test compound was clearly nononcogenic. Historical laboratory data on mammary adenocarcinomas were available to assess that the significant increase in the incidence of this tumor in some dosed groups was not biologically important.

However, the evidence for use of a maximum tolerated dose was weak; there was no decrease in mean body weights in dosed females throughout the study, and the mean weight gains in males were only significantly lower than controls during the first 13 weeks of the study. Mean body weights at 13 weeks were 10 percent lower in the 500-ppm group of males (36.6 ± 4.1) than in controls (40.8 ± 3.2), but at week 104 they were only 2.4 percent lower (44.5 ± 3.9) than in the controls (45.6 ± 9.3). Mean body weights in males receiving 20 and 100 ppm were higher than the controls throughout the study.

There were no toxicologically important effects on mortality, food consumption, clinical laboratory parameters, organ weights, or gross histopathologic findings. The authors based their LOEL for systemic chronic toxicity on clinical observations of increased incidence of piloerection and hunched appearance of animals (males receiving 100 ppm and females receiving 500 ppm). However, if these findings are considered toxicologically important, a LOEL based on data for piloerection should be set at 20 ppm, the lowest dose tested (see data for females at 26, 52, and 78 weeks, Table 1). Therefore, a NOEL was not achieved.

We assess that a tentative LOEL should be based on the decreased weight gain in males at 500 ppm and the NOEL should be set at 100 ppm.

No rationale for dose selection was presented in the report. Because there is only a decreased weight gain in males receiving 500 ppm for the first 13 weeks of the study, it is suggested that the sponsor provide more data that will clarify if the dose chosen for a maximum tolerated dose had adequate rationale.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Method, CBI pp. 2-11.