(EXCERPT)
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DATA EVALUATION RECORD

CYHALOTHRIN

90-Day Feeding Study in Rats

STUDY IDENTIFICATION: Lindsay, S., Chart, I. S., Godley, N. J., Gore, C. W., Hall, M., Pratt, I., Robinson, M., and Stonard, M. Cyhalothrin: 90-day feeding study in rats. (Unpublished study No. PR 0405 and report No. CTL/P/629 by Central Toxicology Laboratory, Imperial Chemical Industries, Ltd., Alderley Park, Macclesfield, Cheshire, U.K. for Imperial Chemical Industries, Ltd., PLC, Alderley Park, Macclesfield, Cheshire, U.K., date of issue: July 24, 1981.) Accession No. 073204.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 9-3-85

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1. CHEMICAL: Cyhalothrin (Grenade): [(RS)α cyano-3-phenoxybenzyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate].

2. TEST MATERIAL: The test material had a pyrethroid content of 92.2% w/w of which 96.8% w/w was cyhalothrin. The batch number was ADM/46156/80. The CTL reference number was Y00102/010/005.

3. STUDY/ACTION TYPE: Subchronic (90-day) feeding study in rats.

4. STUDY IDENTIFICATION: Lindsay, S., Chart, I. S., Godley, N. J., Gore, C. W., Hall, M., Pratt, I., Robinson, M., and Stonard, M. Cyhalothrin: 90-day feeding study in rats. (Unpublished study No. PR0405 and report No. CTL/P/629 by Central Toxicology Laboratory, Imperial Chemical Industries, Ltd., Alderley Park, Macclesfield, Cheshire, U.K. for Imperial Chemical Industries, Ltd., PLC, Alderley Park, Macclesfield, Cheshire, U.K., date of issue: July 24, 1981.) Accession No. 073204.

5. REVIEWED BY:

Robert Weir, Ph.D.
Principal Author
Dynamac Corporation

Signature: Robert Weir
Date: 9/3/85

Finis Cavender, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Finis Cavender
Date: 9/3/85

6. APPROVED BY:

William L. McLellan, Ph.D.
Subchronic Toxicity
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan
Date: 9/3/85

Pamela Hurley, Ph.D.
EPA Reviewer

Signature: Pam Hurley
Date: 1/23/86

Edwin Budd
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

Groups of 20 male and 20 female Wistar-derived rats were fed diets containing 0, 10, 50, or 250 ppm for 90 days.

Body weight gain was significantly reduced in males fed cyhalothrin at 250 ppm. Body weight gain was also significantly reduced in females at this level, but only during the first week. Body weight gain was not significantly affected at lower dosages. Therefore, the LOEL is 250 ppm and the NOEL is 50 ppm for cyhalothrin in rats.

9. CLASSIFICATION: Core Guideline.

Items 8 and 10--See footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A copy of the study author's materials and methods section is appended (Appendix A). A synopsis of the materials and methods follows:

A. Materials and Methods:

1. The test material was technical grade containing 92.2% w/w pyrethroids of which 96.8% was cyhalothrin. One batch (ADM/46156/80) was used for the entire study.

2. The test animals were Wistar-derived rats of the Alderley Park Strain (Specific Pathogen Free). They were acclimated, randomized, uniquely identified, and started on the test diet at approximately 5 weeks of age. Rats were housed 4 per cage by sex and according to dosage group, in stainless steel cages elevated above the droppings.

3. The diets were prepared from Porton Combined Diet supplied by B.P. Nutrition Ltd., Witham, Essex, U.K. The test diets were prepared by mixing appropriate quantities of cyhalothrin with the feed and forming pellets. Control diet was also in pellet form. The dietary concentrations were 0, 10, 50, or 250 ppm cyhalothrin.

4. Dietary homogeneity and stability in pelleted diets were determined. Batches of diets were analyzed for cyhalothrin concentration. Diets were acetone extracted in a Soxhlet apparatus. Following Florisil column clean-up, the extract was analyzed by gas chromatography using an electron capture detection.

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[1]Only items appropriate to this DER have been included.

5. Statistical methods used consisted of analysis of variance, analysis of covariance, Student's t-test, or a one degree of freedom comparison (f-test, equivalent to a t-test). Adjustment for missing values or transformations were used as required.

B. Protocol: (See appended Materials & Methods).

No protocol was included in the report.

12. REPORTED RESULTS:

A. Test Material: Most of the diets analyzed for cyhalothrin content were within 8% of the nominal levels. One premix was incorrectly calculated in correcting for purity and all dosage groups were as much as 26% low for 9 days when the analytical results were reported. Homogeneity was shown to be within ±7% of the overall mean concentration in the diet. The cyhalothrin in the pellets was stable for at least 11 weeks.

B. Survival and Clinical Health: Two female rats from the control group died, one in week 1 and the other in the final week of study. No other deaths occurred. Aside from a scaly tail condition which occurred from approximately the 9th week of treatment to termination, no other effect was noted. The incidence of rats with this finding was similar among groups.

C. Body Weight: There was a reduction in body weight gain in the males at all three dosages throughout the study which was statistically significant only at the 250 ppm level. Females showed lower body weight gains at the 250 ppm level but this effect was only statistically significant in the first week of dosing as shown in Table 1.

D. Food Consumption and Utilization: Males fed cyhalothrin generally consumed less food than control rats. This was only statistically different (lower) than the control group in the 50 ppm group at weeks 6 and 8 and in the 250 ppm group at weeks 1 and 8. In the females, food consumption was reduced in the 250 ppm group during week 1 only. There were no effects on food utilization in either sex at any dosage level.

E. Food Wastage: Food wastage was greater in males fed 50 and 250 ppm cyhalothrin, than the controls, for the first 8 weeks of the study. From week 10 on, there was no compound-related effect on food wastage. Food wastage for the entire 13-week study was greater in the 50 and 250 ppm groups, when compared to the controls, but was statistically significant only in the 50 ppm group. In the females, food wastage did not occur during the first eight weeks of the study and from week 8 to termination lower wastage was seen in the 50 and 250 ppm groups. In the 50 and 250 ppm groups, food wastage was reduced for the entire 13-week study as compared to the controls.

TABLE 1. Selected Body Weight Data for Rats Fed Cyhalothrin for 90 Days

Dietary Concentration (ppm)	Mean Body Weight (g) at Week 0	1	2	7	13	Total Weight gained (g)
Males						
0	136	186	245	414	507	371
10	133	180	236	402	483	350
50	137	182	237	404	495	359
250	134	156**	213**	383**	456**	322**
(Percent of Control)	(99)	(84)	(87)	(93)	(90)	(87)
Females						
0	114	149	176	252	275	161
10	116	150	177	251	274	158
50	113	149	177	248	274	161
250	106	135*	167	235	258	252
(Percent of Control)	(93)	(91)	(95)	(93)	(94)	(94)

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

F. Hematology: The mean red blood cell volume was reduced in all treated groups at week 13. There was also evidence of compensatory increases in red cell counts of all treated groups although they had normal hematocrit and hemoglobin level. At week 4, the mean hemoglobin of female rats fed 250 ppm was reduced slightly; it was also reduced in the 10 ppm group females and the 250 ppm males fed 250 ppm cyhalothrin at week 13. The female 250 ppm group had increased hemoglobin at week 13. No other compound-related hematologic effects were evident. These results are summarized in Table 2.

G. Clinical Chemistry: No changes were found in plasma glucose, albumin, and total protein, levels or in alkaline phosphatase activity. Plasma alanine transaminase, asparatate transaminase activities, and cholesterol levels were statistically significantly increased in the males fed 10 and 50 ppm cyhalothrin after 4 weeks. Plasma alanine transaminase activity was increased in the female 10 ppm group after 4 weeks. Males fed 10 ppm cyhalothrin showed increased plasma urea after 4 weeks, while the 50 ppm male group showed decreased plasma urea levels after 13 weeks. There was a reduction in plasma triglyceride levels at 4 weeks for males fed 250 ppm; at 13 weeks triglyceride levels were decreased in rats fed 50 and 250 ppm cyhalothrin. These results are summarized in Table 3.

H. Urinalysis: There were no differences seen in urine volume, pH, specific gravity, proteins, ketones, or urobilinogen in cyhalothrin-treated groups when compared to the control group. There were small, but statistically significant, differences in male glucose values in the 50 and 250 ppm groups at 13 weeks. Values were as follows:

Urinary Glucose Level for Male Rats at Week 13

	Dietary Concentration (ppm)			
	0	10	50	250
mg/18 hours	0.550	0.650	0.820*	0.930**

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

I. Hepatic Aminopyrine-N-Demethylase Activity (APDM)

At 13 weeks, a dose-related increase (46-68%) in mean APDM activity (μmol HCHO formed g liver/hour) was noted in both sexes at 250 ppm and the males at 50 ppm (34.1%) as compared to the mean control values. Based on log transformation of the data, these increases were significantly different from control mean values at a p of 0.01 using a two-sided t-test (Table 4).

TABLE 2. Selected Hematology Data for Rats Fed Cyhalothrin for 90 Days[a]

	Dietary Concentration (ppm)															
	0				10				50				250			
Weeks	Hb[b] (g/dl)	Hcrit (%)	RBC count ($\times 10^{12}$/l)	Cell vol (fl)	Hb (g/dl)	Hcrit (%)	RBC count ($\times 10^{12}$/l)	Cell vol (fl)	Hb (g/dl)	Hcrit (%)	RBC count ($\times 10^{12}$/l)	Cell vol (fl)	Hb (g/dl)	Hcrit (%)	RBC Count ($\times 10^{12}$/l)	Cell vol (fl)
MALES																
0	13.42	36.5	6.00	60.9	13.34	36.7	6.09	60.5	12.98	35.4	5.86	60.7	13.24	36.5	6.03	60.8
4	15.55	42.3	7.66	55.8	15.74	42.9	7.82	55.5	15.48	42.2	7.70	55.5	15.44	41.9	7.66	55.0
13	15.69	44.0	8.58	52.7	15.91	44.3	8.98*	50.6**	15.46	42.9	8.68	50.9*	15.39	42.5	8.74	49.8**
FEMALES																
0	13.04	36.6	6.05	60.6	12.86	35.9	5.98	59.9	13.21	36.9	6.04	61.1	14.22	39.5	6.53	60.4
4	15.49	43.0	7.40	59.2	15.16	42.3	7.38	59.1	15.44	42.8	7.30	59.8	15.23	41.9	7.25	58.8
13	15.43	43.2	7.79	56.8	15.28	42.7	8.01	55.0	15.54	42.9	7.92	54.6*	15.76	43.5	8.11	55.0*

[a]Statistical analyses of the data used Analyses of Covariance to adjust for differences in pre-exposure values.

[b] Hb = hemoglobin; Hcrit = hematocrit; RBC count = red blood Cell count; cell vol = mean cell volume.

* Significantly different from control value ($p \leq 0.05$).
** Significantly different from control value ($p \leq 0.01$).

TABLE 3. Selected Clinical Chemistry Data for Rats Fed Cyhalothrin for 90 Days[a]

Weeks	Dietary Concentration (ppm)																			
	0					10					50					250				
	Alan trans[b] (mU/ml)	Aspart trans (mU/ml)	Chol (mg/dl)	TriG (mg/dl)	P Urea (mg/dl)	Alan trans (mU/ml)	Aspart trans (mU/ml)	Chol (mg/dl)	TriG (mg/dl)	P Urea (mg/dl)	Alan trans (mU/ml)	Aspart trans (mU/ml)	Chol (mg/dl)	TriG (mg/dl)	P Urea (mg/dl)	Alan trans (mU/ml)	Aspart trans (mU/ml)	Chol (mg/dl)	TriG (mg/dl)	P Urea (mg/dl)
MALES																				
0	16.4	50.2	47.6	114	28.7	16.2	47.6	47.7	94	31.8	15.1	43.2	49.6	95	31.2	13.3	42.7	48.3	81	31.8
4	14.6	35.7	42.4	144	38.4	16.6*	40.7*	47.8**	148	47.1**	17.6**	42.5**	47.1**	167	42.3	15.3	35.5	44.6	112	42.8
13	14.6	45.6	47.2	203	40.8	14.2	45.7	48.6	189	39.7	16.6	48.5	48.3	117**	34.8*	14.7	40.3	44.7	83**	37.8
FEMALES																				
0	12.6	38.7	54.5	77	35.6	13.1	41.7	50.8	80	31.4	14.0	40.7	51.3	68	30.7	11.3	37.6	59.2	91	45.5
4	11.3	35.8	41.3	77	59.0	13.4*	34.2	40.9	74	51.9	12.1	34.8	41.0	96	53.5	12.5	34.9	40.5	61	47.9
13	11.9	38.0	40.0	82	46.8	10.9	45.8	39.3	73	44.8	11.7	34.0	40.0	94	43.8	11.5	36.6	37.7	80	44.8

[a]Statistical analyses of the data used Analyses of Covariance to adjust for differences in pre-exposure values.
[b]Alan trans = plasma alanine transaminase; Aspart trans = plasma aspartate transaminase; Chol = cholesterol; TriG = triglycerides; P Urea = Plasma urea.

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

TABLE 4. Group Mean Hepatic Aminopyrine-N-Demethylase (Week 13)

	µmol HCHO/g liver/hr at a dietary level (ppm) of			
	0	10	50	250
Males	22.6	25.2	30.3**	38.0**
Females	16.9	16.5	17.4	24.7**

**Significantly different from control value ($p < 0.01$) when log transformed data were analyzed.

J. Ophthalmoscopy: Feeding cyhalothrin to rats at 0, 10, 50, or 250 ppm produced no evidence of effect on the eyes of the rats examined.

K. Organ Weights: Organ-weight data are reported in Table 5 for organs where statistically significant results were found. Data are presented as organ weights and organ weights corrected for body weight. A decrease in mean liver weight was seen in the 250 ppm male group. The mean lung weights were slightly, but significantly, decreased for the male and female 250 ppm groups ($p < 0.05$). However, they were not significantly different from control mean values when the mean values were adjusted for body weight. The authors did not explain how the organ weights were adjusted; their statistical analysis used body weights in analyses of covariance with organ weights. When individual liver-to-body weight ratios were calculated (by our reviewers) and analyzed statistically, no significant differences were noted (Table 5). The mean heart weight (adjusted for body weight) was increased in males fed 50 and 250 ppm cyhalothrin. This finding was only statistically significant in the male 50 ppm group. Mean brain weights were slightly decreased in both sexes at the 250 ppm level and in the 10 ppm male group. These differences were partly explained by differences in body weight between the control and treated groups. There was no effect on the kidney, adrenal, gonad, or pituitary weights in either sex.

L. Histopathology: Two female rats from the control group died or were killed moribund during the study. The rat killed during week 1 and the one which died during the 13th week of treatment had pyelonephritis or urolithiasis. The tissues of rats killed at termination had a variety of background histopathologic changes, none of which appeared to be compound related.

M. Electron Microscopy: Mild proliferation of smooth endoplasmic reticulum (SER) was seen in three male rats receiving 50 ppm and three males receiving 250 ppm cyhalothrin; however, the quantitated group means were slightly higher, but not significantly different, from the control group.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Cyhalothrin showed a definite toxicological effect, as judged by a reduction in body weight gain in males receiving 250 ppm as compared to their controls. At 10 and 50 ppm cyhalothrin, the changes "which were accompanied by lower food consumption but no effects on food utilization" were considered to have "resulted from a reduced diet palatability due to addition of cyhalothrin" and to be of no toxicological significance. Therefore, the no-effect level achieved in this study was 50 ppm cyhalothrin.

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TABLE 5. Selected Organ Weight Data for Rats Fed Cyhalothrin for 90 Days

	Males Dietary Concentration (ppm)				Females Dietary Concentration (ppm)			
	0	10	50	250	0	10	50	250
Liver (g)	18.3	17.6	17.6	17.0*	9.7	9.7	9.8	9.6
Adj. Bd. wt.[a]	17.7	17.7	17.3	17.9	9.5	9.6	9.7	10.1
Liver/body wt. ratio(%)[b]	3.65	3.65	3.54	3.73	3.55	3.53	3.58	3.74
Lung (g)	1.69	1.65	1.69	1.60*	1.25	1.25	1.25	1.19**
Adj. Bd. wt.	1.64	1.66	1.67	1.66	1.23	1.24	1.23	1.24
Heart (g)	1.320	1.289	1.365	1.286	0.842	0.869	0.854	0.843
Adj. Bd. wt.	1.288	1.293	1.350*	1.328	0.831	0.862	0.846	0.866
Brain (g)	2.164	2.125*	2.145	2.128*	2.000	1.994	1.984	1.964*
Adj. Bd. wt.	2.153	2.127	2.146	2.143	1.900	1.988	1.977	1.983

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

[a]Mean adjusted for body weight.

[b]Analysis by our reviewers.

B. The protocol was audited at study initiation; there were 14 procedural audits during the conduct of the study. The draft and final reports were audited against the protocol and recorded results.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. The following parameters were not affected by the inclusion of cyhalothrin in the diet of rats: survival; signs of toxicity; hemoglobin, hematocrit, platelet counts, white blood cell counts, differential white cell counts, and prothrombin time; kaoline-cephalin time, plasma alkaline phosphatase, total protein, albumin, and glucose; urine volume, pH, specific gravity (2 hr. sample), protein, ketones and urobilinogens; spleen, gonad, kidney, adrenal, and pituitary weights; ophthalmoscopy; histopathology viewed with light microscopy, and the condition of the SER in the liver viewed with the electron microscope.

A scaly tail condition was the only sign observed frequently. This is not considered compound related. There was a significant reduction in body weight gain in the males at the 250 ppm level. This correlated with food consumption, as males fed cyhalothrin generally consumed less food than the controls; however, this was only statistically significant at the 50 and 250 ppm level. There was no effect on food utilization in any group. Food consumption was reduced in the 250 ppm female group for the first week only. This was accompanied by a significantly lower body weight in the females for the first week. Dietary palatability and food refusal with concurrent reduced body weight seem to be indicated. Reduced mean red cell volume values in both sexes in all three dosages at 13 weeks followed a dose-effect relationship; however, a downward trend was also observed in the controls. Hemoglobin, hematocrit, and red blood cell counts were elevated indicating an opposite trend or an accommodation. Small isolated differences in plasma alanine transaminase, asparatate transaminase, urea, cholesterol, triglycerides, and urinary glucose were not dose related or recurring on a time basis, or they were not supported by histological alterations. Hence, these changes are not considered compound related.

The hepatic aminopyrine-N-demethylase activity was increased in both sexes at the 250 ppm level and in the males at 50 ppm. This is a reversible, compensatory change usually considered to be adaptive rather than toxicological.

B. There are no substantive differences between the authors' and the reviewers' conclusions.

C. The study design and reporting are representative of 90-day subchronic studies conducted in most toxicology laboratories today. During the 9 days when the compound doses in the diets were as much as 26% below nominal, an effect on body weight at lower

levels could have been produced; this effect might not be apparent from the way the study was conducted. When young (weanling) animals are placed on a feeding study, the quantity of food eaten is greater than later in life. Therefore, the dose on a mg/kg of body weight basis would be higher in young animals. In the current study, the initial miscalculated dietary concentration may have affected dietary intake. Nervertheless, the group mean intake of cyhalothrin for the first week of the study was nearly equal in mg/kg/week to that of the second week. The occurrence of the reduced compound intake in the study probably did not adversely affect the study's validity.

Item 15 - see footnote 1.

16. APPENDIX: Appendix A, Material and Methods, CBI pp 2-11.