05/04/2001

EPA Reviewer: Pamela M. Hurley
Registration Action Branch 2 (7509C)

Pamela M. Hurley Date 5/4/2001

DATA EVALUATION RECORD
Supplement to DER for MRID No.: 00153029 Cyhalothrin: 28-Day Feeding Study. **This supplement includes a revised executive summary and supporting tables.**

STUDY TYPE: 28-Day Feeding Study in Rats
OPPTS Number: N/A
OPP Guideline Number: § N/A

DP BARCODE: N/A
P.C. CODE: 128867, 128897
SUBMISSION CODE: N/A
TOX. CHEM. NO.: 271F, 725C

TEST MATERIAL (PURITY): Cyhalothrin Technical (89.0% a.i.)

SYNONYMS: [(RS) α-cyano-3-phenoxybenzyl (z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate]

CITATION: Tinston, D.; Banham, P.; Chart, I.; et al. (1984) PP563: 28-day Feeding Study in Rats: Summary Report: CTL Study No. PR0337: Report No. CTL/P/1056. Unpublished study prepared by Imperial Chemical Industries, PLC. 79 p. MRID 00153029

SPONSOR: Imperial Chemical Industries PLC, Macclesfield, Cheshire, UK

EXECUTIVE SUMMARY: In a 28-day feeding study in male and female SPF Alpk/AP Wistar-derived rats (16/sex/dose), cyhalothrin (PP563, 89.0%) and PP654, an isomer mixture similar to cyhalothrin which contains both cis and trans isomers (cyhalothrin contains only the cis isomer) were fed in the diet at levels of 0, 20, 100, 250, 500 or 750 ppm (estimated to be approximately 0, 2, 10, 25, 50 or 75 mg/kg/day cyhalothrin based on use of very young animals; clinical signs upon which NOAEL is based started on day 3) and 500 or 750 ppm (approximately 50 or 75 mg/kg/day PP564). The animals were examined once daily for clinical signs of toxicity. Bodyweights, food consumption, hematological and clinical chemistry parameters, ophthalmological examinations, urinalysis parameters, organ weights, and macroscopic examinations were conducted and/or measured. For cyhalothrin, livers from up to 8/sex/group were fixed in formol corrosive for microscopic examination. The remaining livers plus selected tissues (including sciatic nerves, brain and spinal cord) from 8/sex/group were fixed in formol saline for microscopic examination. The livers from the PP564 animals were included in this group. In addition, the left sciatic and posterior tibial nerves from 4 male and 4 female controls and high dose cyhalothrin groups were fixed in formol glutaraldehyde for microscopic examination. With all remaining animals, only abnormal appearing tissues were examined microscopically. Livers from 6/sex/group were taken for measurement of hepatic aminopyrine-

N-demethylase (APDM) activity and electron microscopy. Smooth endoplasmic reticulum (SER) was quantified.

At 20 ppm and above, a dose-related increase in APDM activity was observed in males. At 20 ppm, the increase was only slight (26.00 versus 22.30 µmoles HCHO/hr/g liver). Slight hypersensitivity to touch was observed in 4 females starting on day 2; however, this had a variable dose-response. At 100 ppm and above, a dose-related increase in APDM activity was observed in females. At 100 ppm, the increase was only slight (14.21 versus 12.03 µmoles HCHO/hr/g liver). Clinical signs included high stepping gait in 1 male on day 3 and slight hypersensitivity to touch (2 males on days 2-4, 3 females on day 2) and sound (2 males on day 23; again, variable dose-response). At 250 ppm, 1 male and 2 females had high-stepping gait starting on day 2, 2 males had ataxia starting on day 3, 3 males had hunched posture starting on day 4 and 5 females had increased activity starting on day 4. In addition, significant decreases in mean body weight gain and food consumption (both sexes), increases in mean relative liver weights and decreases in mean heart weights were observed at 250 ppm and above. At 500 ppm and above, high stepping gait, ataxia, hunched posture, tail erect, increased activity, lack of grooming and salivation were the major dose-related clinical signs with cyhalothrin. Reductions in serum plasma triglyceride levels and protein excretion levels in urine were observed in males. At higher dose levels, the reductions in serum plasma triglyceride levels were observed in both sexes. With PP564, high stepping gait, ataxia, hunched posture and increased activity in females were observed, but to a lesser extent. Reductions in serum plasma triglyeride levels were also observed. At 750 ppm an additional clinical sign of loss of stability was observed in 1 male and 3 females. With PP564, similar clinical signs were observed as with cyhalothrin, but to a lesser extent. Loss of stability was not observed.

The NOAEL for cyhalothrin is 20 ppm (2 mg/kg/day) and the LOAEL is 100 ppm (10 mg/kg/day) based on clinical signs of neurotoxicity. At higher dose levels, decreases in body weight gain and food consumption and changes in organs weights were also observed. The NOAEL for PP564 is less than 500 ppm (50 mg/kg/day).

This study is classified as **acceptable nonguideline** and does not satisfy any particular guideline requirement.

Incidence of Selected Clinical Observations								
		PP563: Cyhalothrin					PP564	
Dose (ppm) Observation	Control	20	100	250	500	750	500	750
Males								
Convulsions								
High-stepping gait	0/0[a]	0/0	1/1	1/1	46/8	69/8	5/3	33/8
Ataxia	0/0	0/0	0/0	2/2	63/8	133/8	2/1	66/8
Piloerection	22/5	25/5	15/4	31/9	62/8	101/8	31/5	42/8
Hypersensitivity to touch	0/0	0/0	2/2	0/0	6/5	15/4	1/1	1/1
Hypersensitivity to sound	0/0	0/0	2/2	13/9	17/8	82/8	4/4	33/8
Hunched	0/0	0/0	0/0	5/3	51/7	51/8	8/4	51/7
Loss of stability	0/0	0/0	0/0	0/0	0/0	1/1	0/0	0/0
Tail erect	0/0	0/0	0/0	0/0	3/3	23/8	0/0	0/0
Increased activity	0/0	0/0	0/0	0/0	33/8	23/5	0/0	8/3
Decreased activity	0/0	0/0	0/0	0/0	9/4	1/1	0/0	7/6
Ungroomed, stained tail, staining of ventral fur	0/0	0/0	0/0	0/0	4/4	10/4	0/0	0/0
Salivation	0/0	0/0	0/0	0/0	3/2	19/7	0/0	4/1
Weak	0/0	0/0	0/0	0/0	0/0	4/1	0/0	0/0

Incidence of Selected Clinical Observations								
		PP563: Cyhalothrin					PP564	
Dose (ppm) Observation	Control	20	100	250	500	750	500	750
Females								
Convulsions					0/0	6	0/0	0/0
High-stepping gait	0/0	0/0	0/0	4/2	50/8	81/8	1/1	28/8
Ataxia	0/0	0/0	0/0	0/0	23/8	118/8	2/2	26/8
Piloerection	11/5	9/3	10/4	17/4	33/8	98/7	9/4	12/6
Hypersensitivity to touch	0/0	4/4	3/3	16/8	13/5	11/6	2/2	8/3
Hypersensitivity to sound	0/0	0/0	0/0	16/8	6/4	53/6	0/0	18/6
Hunched	0/0	3/1	0/0	0/0	16/6	87/8	4/2	16/5
Loss of stability	0/0	0/0	0/0	0/0	0/0	6/3	0/0	0/0
Tail erect	0/0	0/0	0/0	0/0	1/1	21/8	1/1	15/8
Increased activity	0/0	0/0	0/0	9/5	55/8	15/4	22/4	22/8
Decreased activity	0/0	0/0	0/0	0/0	1/1	5/4	0/0	0/0
Ungroomed, stained tail, staining of ventral fur	0/0	0/0	0/0	0/0	0/0	59/5	0/0	0/0
Salivation	0/0	0/0	0/0	0/0	0/0	33/6	0/0	0/0
Weak	0/0	0/0	0/0	0/0	0/0	33/4	0/0	0/0

Incidence of Selected Clinical Observations								
		PP563: Cyhalothrin					PP564	
Dose (ppm) Observation	Control	20	100	250	500	750	500	750
Depressed respiration	0/0	0/0	0/0	0/0	0/0	4/4	0/0	0/0

[a]Total number of observations in x number of animals

Overall Group Mean Bodyweight Gain (g), Food Consumption and Food Utilization[a]								
		PP563: Cyhalothrin					PP564	
Dose (ppm)	Control	20	100	250	500	750	500	750
Males								
Mean Body Weight Gain/Group	194.1	194.4	192.8	174.9* (90)	147.4* (76)	72.9** (38)	169.4	131.5** (68)
Mean Total Body Weight Gain/Cage	776.5	777.5	771.0	699.8	569.0*	384.0**	657.0	546.5**
Mean Food Consumption/Cage	2516.0	2637.5	2540.7	2233.7* (89)	1947.0* (77)	1148.0** (46)	2311.5 (92)	1886.0** (75)
Mean Food Utilization	3.2	3.4	3.3	3.2	3.4	3.0	3.5	3.5

Overall Group Mean Bodyweight Gain (g), Food Consumption and Food Utilization[a]								
		PP563: Cyhalothrin					PP564	
Dose (ppm)	Control	20	100	250	500	750	500	750
Females								
Mean Body Weight Gain/Group	102.9	101.5	102.9	87.9** (85)	73.9** (72)	42.6** (41)	84.8* (82)	71.0** (69)
Mean Total Body Weight Gain/Cage	411.5	406.0	411.5	351.8*	287.5*	17.2**	332.5	290.5*
Mean Food Consumption/Cage	2035.0	1992.2	1980.2	1726.7* (85)	1591.0** (78)	886.0** (44)	1848.5* (91)	1638.0* (80)
Mean Food Utilization	4.9	4.9	4.8	4.9	5.5	5.2	5.6	5.6

* $p < 0.05$
** $P < 0.01$
[a]Food utilization = Mean food intake per g body weight gained
( ) = % of control

Mean Plasma Triglyceride Levels (mg/100 ml)								
		PP563: Cyhalothrin					PP564	
Dose (ppm)	Control	20	100	250	500	750	500	750
Males								
Value Std. Dev.	185 41	203 60	168 67	142 60	75 20	37 9	115 30	93 44
Females								
Value Std. Dev.	139 31	129 53	143 35	149 36	101 36	45 13	119 49	121 40

Mean Protein Levels in Urine (mg/rat)								
		PP563: Cyhalothrin					PP564	
Dose (ppm)	Control	20	100	250	500	750	500	750
Males								
Value Std. Dev.	24.4 3.7	28.5 7.6	27.8 3.2	23.7 3.6	18.0 5.9	8.7 3.2	24.8 3.0	20.7 6.5
Females								
Value Std. Dev.	1.5 1.3	1.1 0.2	1.2 0.6	1.2 0.3	0.6 0.1	0.9 0.2	0.9 0.2	1.3 0.7

Means of Selected Organ Weights								
		PP563: Cyhalothrin					PP564	
Dose (ppm)	Control	20	100	250	500	750	500	750
Males								
Liver Absolute Relative	15.3 14.3	15.6 14.3	15.9 14.4	15.4 15.1**	14.1* 15.2**	9.4** 13.8	14.6 14.7	12.7** 15.0**
Heart Absolute Relative	1.07 1.03	1.03 0.98	1.02 0.97	0.95** 0.93*	0.86** 0.88*	0.64** 0.76**	0.90** 0.89**	0.85** 0.91*
Kidney Absolute Relative	2.39 2.22	2.33 2.17	2.34 2.17	2.21* 2.12	2.08** 2.16	1.63** 2.08	2.12** 2.08	1.97** 2.19
Spleen Absolute Relative	0.81 0.74	0.75 0.68	0.75 0.68	0.71* 0.67	0.60** 0.63*	0.40** 0.59*	0.69** 0.67	0.57** 0.66
Testes Absolute Relative	2.86 2.71	2.90 2.75	2.86 2.70	2.87 2.80	2.86 93*	2.74 3.14**	2.73 2.69	2.76 2.96*
Brain Absolute Relative	1.93 1.88	1.89 1.85	1.92 1.88	1.94 1.92	1.91 1.93	1.83* 1.95	1.92 1.91	1.84* 1.90

Means of Selected Organ Weights								
		PP563: Cyhalothrin					PP564	
Dose (ppm)	Control	20	100	250	500	750	500	750
Females								
Liver Absolute Relative	9.2 8.7	9.2 8.6	9.6 9.1	9.4 9.6*	8.8 9.4	7.3** 9.6	8.8 9.1	8.8 9.7*
Heart Absolute Relative	0.72 0.68	0.74 0.70	0.75 0.71	0.73 0.74	0.70 0.72	0.52** 0.65	0.74 0.74	0.66 0.70
Kidney Absolute Relative	1.58 1.51	1.63 1.53	1.63 1.56	1.57 1.58	1.49 1.52	1.35* 1.61	1.52 1.52	1.53 1.62*
Spleen Absolute Relative	0.47 0.46	0.46 0.44	0.51 0.49	0.44 0.44	0.45 0.46	0.28** 0.33*	0.45 0.45	0.49 0.50
Ovaries Absolute Relative	0.129 0.125	0.145 0.141	0.124 0.121	0.107 0.108	0.116 0.119	0.072** 0.086*	0.102* 0.103	0.090** 0.094*
Brain Absolute Relative	1.76 1.75	1.78 1.76	1.76 1.75	1.77 1.77	1.80 1.80	1.56** 1.60*	1.77 1.77	1.78 1.79

* $p < 0.05$, ** $p < 0.01$, ( ) = % of control

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

005316

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral (82-1) rat TOX. CHEM. NO.: 271F

ACCESSION NUMBER: 073980

TEST MATERIAL: (RS)alpha-cyano-3-phenoxybenzyl (Z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate and (RS)alpha-cyano-3-phenoxybenzyl (EZ)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylate

SYNONYMS: Cyhalothrin, PP563 (active ingredient of Grenade) for first test chemical and PP564 for second test chemical

STUDY NUMBER(S): PRO337

REPORT NUMBER: CTL/P/1056

SPONSOR: ICI PLC Plant Protection Division, UK

TESTING FACILITY: ICI PLC, Cntrl. Tox. Lab., Alderly Park, Macclesfield, UK

TITLE OF REPORT: PP563: 28-Day Feeding Study in Rats - Summary Report

AUTHOR(S): Tinston DJ, Banham PB, Chart IS, Gore CW, Pratt I, Scales MDC, Weight TM.

REPORT ISSUED: 7/12/84

IDENTIFYING VOLUME: Volume II, Book 1 of 2, Section C, Tab Ref. 9C

CONCLUSION: For male rats effects were noted at the lowest dose level PP563, 20ppm. For females, the NOEL was 20 ppm. PP564 was less toxic than PP563, indicating that the cis isomer is more toxic than the trans isomer.

Classification: Not Core Guideline, but acceptable for the purposes for which it was performed.

MATERIALS AND METHODS:

Chemical:

PP563 was given the following references: CTL - Y00102/006/001 and Plant Protection Batch P5. It had a purity of 89.0% w/w (100% cis isomer). PP564 was given the following references: CTL - Y00102/001/001 and Plant Protection Batch P5. It had a purity of 84.0% w/w (50:50 cis:trans isomers). Both were viscous, pale yellow liquids.

Animals:

Male and female Alpk/AP (Wistar-derived) rats were obtained from the Animal Breeding Unit at ICI PLC, Alderly Park, Macclestield, Cheshire, UK. The rats were 3 weeks old and were acclimated for one week. The animals were supplied in two groups, one group arriving a week ahead of the other group.

Protocol:

Six groups of 16 male and 16 female rats were fed the experimental diets at the following dose levels for 28 days: 0, 20, 100, 250, 500, and 750 ppm (PP563); and 500 and 750 ppm (PP564). All rats were observed once daily throughout the experimental period for any clinical signs of toxicity. The eyes of all rats from the control, 500 and 750 ppm groups (PP563) were examined pre-experimentally and during the week prior to termination with an ophthalmoscope with and without a mydriate. Bodyweights were recorded weekly and food consumption was recorded daily for the first week and weekly thereafter.

Clinical Chemistry:

The following clinical chemistry parameters were measured in up to 8 designated male and female rats per group prior to the experimental phase and at termination: plasma urea, glucose, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and triglyceride and plasma cholesterol levels (at termination only).

Urinalysis:

Urinalysis measurements were taken from up to 4 male and 4 female rats prior to the experimental phase and at termination. The rats were given an oral water load at 2.5 ml/100g bodyweight and the urinary volume, pH, specific gravity and urinary sediments were measured. The animals were then deprived of water for 18 hours during which time the urine was collected for analysis of urinary volume, pH, specific gravity, protein, glucose, bilirubin and ketones.

Hematology:

The following hematological measurements were taken pre-experimentally and terminally from up to 8 male and 8 female animals per group: hemoglobin, total white cell count, red cell count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, hematocrit, differential white cell count and platelet count. The morphological appearance of the red cells were also examined. At termination, in addition to the above, prothrombin and kaolin/cephalin time tests were conducted and 2 bone marrow smears from the right femurs of all rats were examined for any cytological abnormalities.

Pathology:

Any rats found dead or moribund during the study received a full post mortem examination and tissues were submitted for histopathological examination. The weights of the following organs were recorded from up to 8 male and females rats per group: gonads (combined), spleen, adrenals (combined), kidneys (combined), liver, thymus, heart, lungs (combined), brain and pituitary. The livers from these animals (except the PP564 livers) were fixed in formol corrosive for histopathological examination. The livers from the PP564 group along with the following tissues from 8 male and 8 female animals per group were fixed in formol saline: salivary glands (parotid, sub-maxillary and sub-lingual), cervical lymph node, mammary tissue, voluntary muscle, testes, epididymides, protate and seminal vesicles or ovaries, uterus and cervix, urinary bladder, spleen, pancreas, stomach, duodenum, jejunum, ileum, mesenteric lymph node, caecum, colon, adrenals, kidneys, liver, thyroid, aorta, trachea, esophagus, thymus, heart, lungs, eyes, sciatic nerves, brain and spinal cord. The left sciatic and posterior tibial nerves from 4 male and 4 female controls and 750 ppm PP563 groups were fixed in formol glutaraldehyde and examined. All remaining animals received a gross post mortem examination and only abnormal appearing tissues were submitted for histopathological examination. Livers from a designated 6 male and 6 female animals from all groups were taken for measurement of hepatic aminopyrine-N-demethylase activity. These livers were the same as those taken for measurement of weight and examination by electron microscope. For the electron microscopy, samples were taken from the median lobes from the preselected male and female animals from control, 20, 100 and 250 ppm groups (PP563). Smooth endoplasmic reticulum (SER) was quantified using the point counting method of Weibel.

RESULTS:

Dietary Concentrations:

Concentrations of PP563 and PP564 were within 10% of the nominal values except for the 500 ppm PP563 and 500 ppm PP564 diets where the mean concentrations were 83% and 89% respectively. PP563 was shown to be stable in the diet for up to 30 days after preparation.

Mortalities:

Three male and three female rats receiving 750 ppm PP563 in the diet were found to be either dead or moribund. As a result, a second batch of animals already scheduled to start one week later were fed 500 ppm instead (this also included a second batch of PP564 animals). At 750 ppm, 2 more female rats died, one after 14 days and one after 27 days. No other deaths occurred during the study.

Clinical Observations:

Clinical observations included high-stepping gait, severe ataxia, hypersensitivity to external stimuli, piloerection and excessive salivation at the 750 and 500 (less severe) ppm (PP563) levels and similar but transient effects at the 250 ppm level. At 100 ppm, one male showed high stepping gait on day 3. Also at the lower levels there was occasional evidence of slight hypersensitivity to external stimuli. The clinical effects observed with PP564 were comparable but less severe: the effects noted at the 750 ppm level were similar to those noted at the 500 ppm level of PP563 and the effects observed at the 500 ppm level were similar to those noted at the 250 ppm level of PP563.

Bodyweight Gain and Food Consumption:

Statistically significant decreases in bodyweight gain were noted for male and female groups receiving either PP563 or PP564 at dietary concentrations of 250 ppm or greater (except for bodyweight gains of males receiving 500 ppm PP564). Statistically significant reductions in food consumption were also observed in both male and female rats fed levels of 250 ppm or greater for both PP563 and PP564.

Clinical Chemistry and Urinalysis:

Reductions in plasma triglyceride levels were noted in males receiving either 500 or 700 ppm PP563 and to a lesser extent in females receiving 750 ppm PP563 and males receiving either 500 or 750 ppm PP564. Dose-related decreases in protein excretion levels in the urine were observed in males receiving either 500 or 750 ppm PP563.

Organ Weights:

Statistically significant increases in liver weights (after adjustments for bodyweights) were observed in the 250 and 500 ppm dose groups (PP563) and in the 750 ppm (PP564) dose group. At 750 ppm 563, the large bodyweight reduction distorted the organ weight analysis. There was some evidence of increased testes weights and decreased ovary weights at the 500 and 750 ppm levels of PP563. There was a dose-related reduction in the heart weight of males fed diets containing PP563 which was statistically significant down to 250 ppm. There was also some evidence for reduction in spleen, brain and thymus weights in groups which grew less than controls.

Histopathology:

Male and female rats dying or killed in extremis showed thymic atrophy, and enlargement, vacuolation and differential staining of the cortical cells of the adrenals. In males, incomplete spermatogenesis and reduction of seminal vesicular secretion was evident. No changes in the nervous system were present. No other changes were noted.

Hepatic Aminopyrine Demethylase Activity:

A dose-related increase in APDM activity was observed in male rats receiving 20 ppm and above (PP563), in females receiving 100 ppm and above (PP563) and in PP564 but to a lesser extent.

Electron Microscopy:

There was a statistically significant increase in SER proliferation (greater in males than in females) which did not show any dose-response effect. The effect was observed in males at dose levels of 20, 100 and 250 ppm PP563 and in females at 250 ppm PP563. One female rat receiving 250 ppm PP563 showed marked vacuolation of hepatocyte cytoplasm, as a consequence of dilatation of endoplasmic reticulum.

DISCUSSION:

The results of this study confirmed the results of another previously submitted 28-day study on cyhalothrin in rats (Moyes et al. 1984) conducted at dose levels of 1 - 250 ppm. Clinical observations indicated signs of neurotoxicity, characteristic of synthetic pyrethroid toxicity. Evidence of decreased bodyweight gain and food consumption was also noted, as well as increased ADPM activity and proliferation of SER. As evidenced by comparing the results from testing PP564 with the results from PP563, it appears that the cis component is the more toxic of the 2 isomers. It should be noted that even at the lethal dose of 750 ppm PP563, no histopathological changes were observed in the peripheral nerves, even when accompanied by neurotoxic signs. The liver hypertrophy accompanied by increases in liver weight, APDM activity and SER proliferation are characteristic of effects due to pyrethroid administration. These effects are considered to be adaptive in this case. The authors stated that the histopathological changes noted in the animals that died were due to stress rather than PP563 toxicity, especially since there was no sign of these changes in the animals that survived.

The purpose of the study was to find the highest dose useful for a longer term study and to compare the toxicity of PP563 with PP564. It was recommended that for longer term studies, dosages higher than 250 ppm should not be used. This study is not Core Guideline because the exposure time was only 28 days and only 8 of the animals per sex per dose group were examined for many of the measurements taken. However, the study is acceptable for the purpose that it was conducted.

DER #16

Cyhalothrin: 28-Day Feeding Study in Rats
ICI PLC. 1984. MRID No. 00154806
HED Doc. No. 005100

EPA Reviewer: Pamela M. Hurley
Registration Action Branch 2 (7509C)

Pamela M Hurley . Date 4/11/2001

DATA EVALUATION RECORD
Supplement to DER for MRID No.: 00154806 Cyhalothrin: 28-Day Feeding Study in the rat.
This supplement includes a revised executive summary.

STUDY TYPE: 28-Day Feeding Study - Rat
OPPTS Number: N/A
OPP Guideline Number: § N/A

DP BARCODE: N/A
P.C. CODE: 128867, 128897
SUBMISSION CODE: N/A
TOX. CHEM. NO.: 271F, 725C

TEST MATERIAL (PURITY): Cyhalothrin Technical (89.2% a.i.)

SYNONYMS: [(RS) α-cyano-3-phenoxybenzyl (z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate]

CITATION: Moyes, A.; Godley, M.; Hall, M., et al. (1984) Cyhalothrin: 28-Day Feeding Study in the Rat (Second Study): Summary Report: Report No: CTL/P/1013. Unpublished study prepared by Imperial Chemical Industries PLC. 33 p. MRID 00154806

SPONSOR: Imperial Chemical Industries, PLC, Macclesfield, Cheshire, U.K.

EXECUTIVE SUMMARY:

In an oral toxicity study SPF Wistar (Alderly Park strain) rats (8/sex/dose) were dosed with cyhalothrin (89.2% a.i.) in the diet at 0, 1, 5, 10, 20 or 250 ppm (approximately 0, 0.1, 0.5, 1.0, 2.0 or 25.0 mg/kg/day using a factor of 10 for young animals) for 28 days (MRID 00154806). Animals were examined for clinical signs of toxicity and the following parameters were measured: body weights, liver weights and hepatic aminopyrene-N-demethylase (APDM) activity. In addition, the livers were subjected to electron microscopic examinations.

No effects were observed at 1, 5 and 10 ppm. At 20 ppm and above, a reduction in mean body weight gain was observed in females ($p \leq 0.05$; 22% less than the control value for weeks 0-4); however, body weight was not affected. At 250 ppm, a reduction in mean body weight gain was observed in males (13% less than the control value for weeks 0-4). In addition, increases and/or proliferation in APDM (14-40%) and smooth endoplasmic reticulum (SER) was observed in both sexes. Relative liver weights were increased in males (7%); however, absolute liver weights were not affected. **The NOAEL is 10 ppm (1.0 mg/kg/day in females) and 20 ppm (2.0 mg/kg/day in males) and the LOAEL is 20 ppm (2.0 mg/kg/day in females) and 250 ppm**

(25.0 mg/kg/day in males) based on decreases in mean body weight gain in females at 20 ppm and above and in males at 250 ppm, and increases and/ or proliferation in APDM and SER in in both sexes at 250 ppm.

This study is classified as **acceptable nonguideline** and does not satisfy any particular guideline requirement.

005100

EPA: 68-01-6561
TASK: 107
September 3, 1985

DATA EVALUATION RECORD

CYHALOTHRIN

28-Day Feeding Study in the Rat

STUDY IDENTIFICATION: Moyes, A., Godley, M. J., Hall, M., Pratt, I., Stonard, R. D., Tinston, D. J., and Forbes, D. 28-Day feeding study in the rat. (Unpublished study No. PR 0397 and report No. CTL/P/1013 by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K., for Imperial Chemicals Industries, Alderley Park, Macclesfield, Cheshire, U.K., dated May 15, 1984) Accession No. 073204.

APPROVED BY:

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

Signature: I. Cecil Felkner

Date: 9-3-85

005100

1. CHEMICAL: Cyhalothrin [(RS)α-cyano-3-phenoxybenzyl(z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate].

2. TEST MATERIAL: Viscous dark brown liquid with a 89.2% (w/w) cyhalothrin content. Unspecified as to technical grade or formulation. The CTL reference number was Y00102/010/001.

3. STUDY/ACTION TYPE: Subchronic (28-day) feeding study in rats.

4. STUDY IDENTIFICATION: Moyes, A., Godley, M. J., Hall, M., Pratt, I., Stonard, R. D., Tinston, D. J., and Forbes, D. 28-Day feeding study in the rat. (Unpublished study No. PR 0397 and report No. CTL/P/1013 by Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K., for Imperial Chemicals Industries, Alderley Park, Macclesfield, Cheshire, U.K., dated May 15, 1984) Accession No. 073204.

5. REVIEWED BY:

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6. APPROVED BY:

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Date: 4/21/86

7. CONCLUSIONS:

Feeding cyhalothrin to rats caused a significant decrease in mean body weight gain during the first week of the study in males receiving 250 ppm ($p \leq .05$) and in females receiving 10, 20 ($p \leq .05$), or 250 ($p \leq .01$) ppm. In addition, there was a significant reduction in mean weight gain over the 4 weeks of the study in males receiving 250 ppm ($p \leq .05$) and females receiving 20 or 250 ($p \leq .05$) ppm. Hepatic aminopyrine demethylase activity (HADA) was increased, and smooth endoplasmic reticulum (SER) was proliferated in the livers of rats of both sexes receiving the high dose of cyhalothrin. Liver weights were not significantly affected by the test substance, but liver-to-body weight ratios were higher ($p \leq .01$) in the male 250 ppm group. As defined within the scope of this study, the NOEL for cyhalothrin in female rats is 10 ppm and the LOEL is 20 ppm; and the NOEL in male rats is 20 ppm and the LOEL 250 ppm.

Item 8 - see footnote 1.

9. BACKGROUND:

In a previous 28-day feeding study in rats (Faupel, P. F., et al., 1980), male rats fed 20 ppm cyhalothrin showed a trend towards elevated hepatic aminopyrine-N-demethylase activity at termination. At dietary levels of 20 ppm and above, there was proliferation of hepatic smooth endoplasmic reticulum (SER) in male rats and in the female rats fed 250 ppm cyhalothrin. The present study was designed to establish a no effect level (NOEL) to be used in setting levels for a long-term study.

Item 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The cyhalothrin used in the study was supplied by ICI, Ltd. pharmaceutical division. It was a dark brown viscous liquid with a cyhalothrin content of 89.2% (w/w).

2. The test animals were Wistar derived Alderley Park rats, bred as SPF animals. Dosing started when the animals were 5 weeks old.

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

3. The basal diet was Porton Combined Diet (PCD) manufactured by Special Diets Service. The test substance was applied to the diet as an acetone solution. Pellets were made and air dried in a furnace at 50°C. The dietary dosages of cyhalothrin were control, 1, 5, 10, 20, and 250 ppm.

4. Animals were randomly distributed to experimental groups using a shuffle card method. Body weights, body weight gains, liver weights, ratios, hepatic APDM, and quantified E.M. results were compared, test to control, using a two-sided Student's t-test.

5. Test and control diets were prepared for analysis of cyhalothrin by Soxhlet extraction, cleaned up through Florisil columns and the eluate analyzed by gas-liquid chromatography using an electron capture detector.

B. Protocol:

See Materials and Methods in Appendix A.

12. REPORTED RESULTS:

A. The cyhalothrin content of all but one of the test diets was found to be within ± 10% of the target cyhalothrin content; the 1 ppm diet was 81% of the target cyhalothrin content.

B. No deaths occurred. No signs of toxicity or clinical observations related to the test substance were seen at any dose level throughout the study. Mean body weights and mean body weight gains are presented in Table 1 and Table 2, respectively. There were statistically significant reductions in body weight gains during the first week of study for males and females receiving 250 ppm ($p \leq .01$) cyhalothrin and for the females receiving 10 and 20 ppm ($p \leq .05$). Also, there was a significant reduction ($p \leq .05$) in body weight gain from the start to completion of the study for males and females receiving 250 ppm cyhalothrin and for the females receiving 20 ppm. Mean body weight was significantly reduced ($p \leq .05$) at the 250 ppm level in weeks 1 and 2 of the study. In the males receiving 250 ppm cyhalothrin, liver-to-body weight ratios were increased ($p \leq .01$) while liver weight was lower than the control but not significantly reduced. There was a significant reduction ($p \leq .05$) in liver weight in females receiving 20 ppm cyhalothrin; the liver-to-body weight ratio was not affected. HADA activity was increased ($p \leq .01$) in both sexes receiving 250 ppm cyhalothrin. Mild but statistically significant ($p \leq .01$) pro- liferation of smooth endoplasmic reticulum (SER) in hepatocytes was seen in male and female rats receiving 250 ppm cyhalothrin. A few males in the 20 ppm group also showed SER proliferation but this was not statistically different from control values.

C. Table 3 presents the results of mean liver weights, mean liver-to-body weight ratios, hepatic aminopyrine-N-demethylase activity (HADA), and smooth endoplasmic reticulum measurements (SER).

TABLE 1. Mean Body Weights for Rats Fed Cyhalothrin for 4 Weeks

Week	Dietary Concentration (ppm) 0	1	5	10	20	250
Males						
0	124.9	111.9	118.6	120.0	116.5	117.5
1	181.0	166.5	176.0	176.1	175.4	152.1*
2	233.0	215.4	230.4	228.4	230.8	204.4*
3	278.9	263.0	276.0	273.9	280.9	251.0
4	319.4	296.1 (93)[a]	319.9 (100)	314.4 (98)	323.0 (101)	286.0 (90)
Females						
0	94.6	96.8	106.9	109.6	107.9	104.5
1	142.3	140.8	145.4	142.8	141.0	131.0
2	167.8	164.3	171.8	167.4	163.1	160.1
3	190.0	185.3	196.5	186.6	185.0	182.1
4	210.4	201.9 (96)	215.8 (102)	203.8 (100)	197.9 (94)	197.0 (94)

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

[a]Percent of control.

TABLE 2. Mean Body Weight Gain for Rats Fed Cyhalothrin for 4 Weeks

Week	Dietary Concentration (ppm)					
	0	1	5	10	20	250
Males						
0 - 1	56.1	54.6	57.4	56.1	58.9	34.6**
1 - 2	52.1	48.9	54.4	52.3	55.4	52.3
2 - 3	45.8	47.6	45.6	45.5	50.1	46.6
3 - 4	40.5	33.1	43.9	40.5	42.1	35.0
0 - 4	194.5	184.3	201.3	194.4	206.5	168.5*
Females						
0 - 1	47.6	44.0	38.5	33.1*	33.1*	26.5**
1 - 2	25.5	23.5	26.4	24.6	22.1	29.1
2 - 3	22.3	21.0	24.8	19.3	21.9	22.0
3 - 4	20.4	16.6	19.3	17.1	12.9	14.9
0 - 4	115.8	105.1	108.9	94.1	90.0*	92.5*

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

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

TABLE 3. Selected Liver Data for Rats Fed Cyhalothrin for 4 Weeks

Effect Measured	Dietary Concentration (ppm) 0.0	1.0	5.0	10	20	250
Males						
Liver Weight (g)	15.581	14.364	15.723	15.703	16.323	14.926
Liver/Body Wt. Ratio	4.871	4.852	4.913	4.977	5.049	5.212**
HADA[a]	30.9	30.2	29.5	32.5	30.5	43.9**
SER[b]	134.3	--	--	131.8	146.3	169.7**
Females						
Liver Weight (g)	9.923	9.551	9.988	9.553	8.925*	9.076
Liver/Body Wt. Ratio	4.720	4.727	4.632	4.690	4.508	4.608
HADA	12.6	12.4	12.0	14.1	13.6	17.7**
SER	109.4	--	--	--	105.8	130.9**

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

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

[a] Hepatic Aminopyrine Demethylase Activity expressed as µmol formaldehyde/hour/g tissue.

[b] Smooth Endoplasmic Reticulum.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. "In conclusion, cyhalothrin produced definite toxicological effects at a dietary level of 250 ppm. This level is recommended as the maximum level for a long-term feeding study. The no effect level achieved in this study is 10 ppm cyhalothrin." Principal toxic effects included weight gain suppression and liver toxicity consisting of increased SER proliferation and increased HADA activity.

B. The draft and final reports were audited for good laboratory practice and the methods and results given in the report were felt to reflect the data produced during the study.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. This specific study design was based on results obtained from a prior study in which liver alterations were found. There was no effect on survival at any dosage level. No judgment can be made on signs of toxicity as no data were included. Body weight was statistically decreased ($p \leq .05$) in male rats at 250 ppm for the first 2 weeks. The male 250 ppm group's weight gain was decreased at week one only, while the females' weight gains were decreased at 10, 20, and 250 ppm for week one. When weight gains were examined over the entire study, there was a decrease for the males at 250 ppm and for the females at 20 and 250 ppm. Although no food consumption measurements were taken, it appears that body weight and body weight gains were compound affected early in the study, with accommodation taking place.

The liver is clearly affected due to dietary exposure to cyhalothrin. The significantly reduced liver weight for the female 20 ppm group appears not to follow a dose-effect relationship and does not appear to be compound related. The male rats at 250 ppm showed an increased liver weight-to-body weight ratio, increased HADA, and proliferation of the SER. The female rats at the 250 ppm level showed increased HADA and proliferation of the SER. The SER proliferation occurred without a concommitant increase in liver weight.

B. There are no substantive differences between conclusions reported by the study authors and those of the reviewer.

C. The study was not designed as a core study but as a follow-up to set the NOEL and LOEL for cyhalothrin in rats. As defined within the scope of this study, the NOEL for cyhalothrin in rats is 10 ppm and the LOEL is 20 ppm based on body weight and liver effects.

Item 15 - see footnote 1.

16. CBI APPENDIX:

Appendix A (CBI pp. 2-7) Materials and Methods.

Core Classification: Core supplemental because the design and conduct of the study were so limited.

APPENDIX A

Materials and Methods

(CBI pp. 2-7)

1. INTRODUCTION

Cyhalothrin [(RS)α-cyano-3-phenoxybenzyl)(z)-(1RS,3RS)-3-(2-chloro-3,3,3 trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate] is a synthetic pyrethroid intended for use as an insecticide on animals.

In a previous study (Faupel et al 1980) male rats fed 20ppm cyhalothrin in the diet for 28 days showed a trend towards elevated hepatic aminopyrine-N-demethylase activity at termination. At dietary levels of 20ppm cyhalothrin and above, there was also evidence for a treatment-related proliferation of the hepatic smooth endoplasmic reticulum (SER) in male rats. SER proliferation in females was seen only in rats fed 250ppm cyhalothrin.

The dietary route of administration and the Alderley Park strain of rat were used for this study to allow comparison with a previous 28-day study (Faupel et al 1980).

The present study was designed to establish a no-effect level for cyhalothrin when administered in the diet to rats over a 28 day period. The results will be considered when setting dose levels for a long term rat study. The study started on 15 April 1980 and finished on the 16 May 1980.

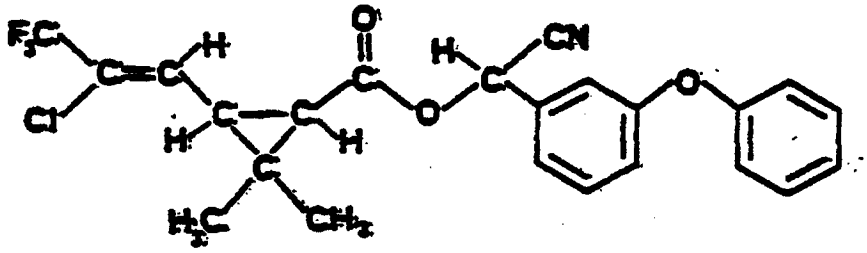
All original data pertaining to this study are stored in the Archives, Central Toxicology Laboratory, Imperial Chemical Industries PLC, Alderley Park, Macclesfield, Cheshire. Copies of the final report are kept in the Reports Centre at Alderley Park.

2. EXPERIMENTAL PROCEDURES

2.1 Test Material

Cyhalothrin: [(RS)α cyano-3-phenoxybenzyl(z)-(1RS,3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2 dimethylcyclopropane-carboxylate].

Chemical structure:



The cyhalothrin used in this investigation was supplied by Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire. The single batch of test compound used in this study was a viscous dark brown liquid with a total cyhalothrin content of 89.2% (w/w). The CTL reference number assigned to this batch of cyhalothrin was Y00102/010/001.

2.2 Diet

All diets were based on Porton Combined Diet (PCD) supplied by Special Diets Services [formerly BP Nutrition (UK) Ltd] Stepfield, Witham, Essex, UK. The diet formulation details are given in Appendix 1. Details of diet preparation are given in Appendix 2.

Control and test diets were analysed for the presence of cyhalothrin using pelleted samples prepared for the study. Details of the method of analysis for cyhalothrin in rodent diet are given in Appendix 3.

2.3 Animals and Accommodation

A total of sixty-five male and sixty-five female Wistar derived Alderley Park rats were supplied by litter, to the Central Toxicology Laboratory Specific Pathogen Free (SPF) Unit from the Animal Breeding Unit, Imperial Chemical Industries Limited, Alderley Park, Cheshire, UK. They were supplied at 21 days of age and were housed under Specific Pathogen Free conditions in a barrier maintained area.

Personnel access to the animal room was restricted for ten days after the arrival of the rats. The clinical condition of the rats was observed once daily for clinical and behavioural abnormalites and for any reaction to the new environment. The rats were acclimatised to their experimental environment for 14 days prior to the start of the study.

On arrival the rats were housed by litters in rat racks supplied by All Type Tools Ltd, Purland Road, Woolwich Industrial Estate, Woolwich, London. The cages were constructed of stainless steel with solid sides. The floor, back and front were constructed of 14 standard wire gauge stainless steel mesh at 1.27cm centres. The internal dimensions were 34x37.5x20.3cm with a floor area of 1275 square cm. Each cage had a removable food hopper of 400g capacity and facility for a 225ml water bottle (North Kent Plastics Ltd) if required. The rack was fitted with an automatic watering system (All Type Tools Ltd) providing the rats with water ad libitum. The cages were suspended over trays lined with absorbent paper sheets.

The temperature of the animal room was maintained within the range 18 to 24°C (as recorded daily by a maximum and minimum thermometer). Relative humidity was maintained within the range 31 to 44%. The lighting was controlled by a time switch giving alternate periods of 12 hours light and 12 hours dark (7am-7pm).

2.4 Experimental Design

Six groups of eight male and eight female rats were fed the experimental diets for 28 days as detailed in Table 1 shown overleaf.

TABLE 1

Group	Dietary Concentration of Cyhalothrin(ppm)	Animal Numbers	
		Male	Female
1	0	1-8	49-56
2	1	9-16	57-64
3	5	17-24	65-72
4	10	25-32	73-80
5	20	33-40	81-88
6	250	41-48	89-96

Ten days into the acclimatisation period and housed in their original litters the rats were randomly assigned to the experimental groups as detailed in Appendix 4.

The groups were arranged on the racks in single sex replicates, each replicate contained one cage of four rats per group. The sequence of distribution of groups within the replicates was determined by a shuffle card method. Individual rats were uniquely identified by ear punch with the experimental number allocated. Details of the distribution of groups and rats on the racks are shown in Appendix 5.

After randomisation the surplus rats were discarded.

At five weeks of age each group of rats was fed their appropriate experimental diet. One replicate of rats was started on study on each day over a four day period during the week beginning 15 April 1980.

3. EXPERIMENTAL INVESTIGATION

3.1 Clinical Observations

Prior to the start of the study all the rats were examined to ensure that they exhibited normal activity. Throughout the study they were checked daily for changes in clinical condition and behaviour and once weekly a detailed examination of each rat was made. Any abnormalities were recorded.

3.2 Bodyweights

Individual bodyweights for all rats in the study were recorded in replicate order prior to initially feeding the experimental diets and weekly thereafter on the same day of each week throughout the study.

4. PATHOLOGY

All rats in the study were subjected to a gross post mortem examination.

4.1 Terminal Investigation

All rats were killed with an overdose of halothane vapour (FLUOTHANE, Imperial Chemical Industries Limited, Macclesfield, Cheshire, UK) and exsanguinated by cardiac puncture to standardise liver weights. The liver of the rats was removed as soon as possible after death, weighed and a section was taken from the median lobe for electron microscopy. The remainder of each liver was placed on ice for determination of hepatic APDM activity.

4.2 Hepatic Aminopyrine Demethylase Activity

Hepatic Aminopyrine-N-Demethylase (APDN) activity was determined for all rats.

DER #17

Cyhalothrin: Metabolism Studies in Rats and Dogs
ICI PLC. 1981,1983,1984, 1985. MRID Nos. 00150852, 00150843, 00151116, 00153036, 00153037, 00153037
HED Doc. Nos. 005100, 011241, 005316

005100

Reviewed by: Pamela Hurley
Section 2 , Tox. Branch (TS-769C)
Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism Study 85-1

ACCESSION NUMBER: 073217

TEST MATERIAL: Cyhalothrin

SYNONYMS: (R,S)alpha-cyano-3-phenoxybenzyl (±)-cis-3-(2-2-chloro-3,3,3-trifluoropropyl-enyl)-2,2-dimethylcyclopropane carboxylate; ICI 146,814; ^{14}CHCN; ^{14}C-cyclopropyl

STUDY NUMBER(S): ICI - 146814 MMR 002/01 and MMR 002/02

REPORT NUMBER: Protocol ICI 146,814 MPH 01

SPONSOR: Imperial Chemical Industries PLC (ICI PLC)

TESTING FACILITY: ICI PLC Pharmaceuticals Division, Safety of Medicines Department

TITLE OF REPORT: Cyhalothrin: The Disposition and Metabolism of ^{14}C-ICI 146,814 In Rats Parts I and II

AUTHOR(S): M. P. Harrison, D. E. Case

REPORT ISSUED: October 8, 1981 and September 17, 1984

IDENTIFYING VOLUME: Volume II, Book 15 of 16 (Tab Reference 19C)

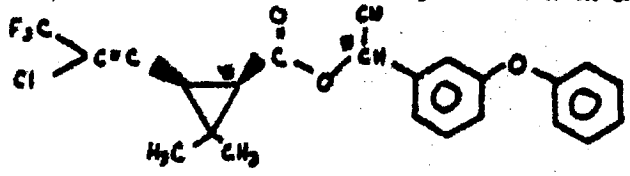
CONCLUSION: This study, in combination with the two following studies, is classified as CORE GUIDELINE. Although there were no indications of any toxic or pharmacologic signs at the highest dose level, the studies were extremely well done and complete.

Classification: CORE GUIDELINE

MATERIALS AND METHODS:

Chemical

Two different radiolabelled forms of cyhalothrin were used for these studies. The positions of radiolabelling are shown in the following figure:



The abbreviations "^{14}CHCN" and "^{14}C-cyclopropyl" were used to refer to the compound labelled at positions marked * or *, respectively, as shown above. Several batches of each were prepared by the Radiochemical Unit of the Drug Metabolism Section at ICI Pharmaceuticals Division and were purified by HPLC. The material used was greater than 99% pure cis isomer, and a racemic mixture of the other possible isomers. Non-labelled cyhalothrin of comparable purity was used for dilutions.

Animals

Male and female 'Alderly Park' Wistar strain 'Specific Pathogen Free' rats weighing between 200-250 grams were used for the studies.

Single Dose Excretion Studies

Three single dose excretion studies were conducted: two oral administration studies (one each with one of the two radiolabelled compounds), and one subcutaneous injection study with only ^{14}CHCN. Six male and six female rats were tested in each study, the dose levels having been set at 1 and 25 mg/kg for the oral studies and 1 mg/kg for the subcutaneous study. For dosing at 1 mg/kg, each ^{14}C compound was dissolved at approximately 0.5 mg/ml in corn oil and for dosing at 25 mg/kg, the ^{14}C compounds were mixed 1:24 w/w with non-labelled cyhalothrin and dissolved in corn oil at 12.5 mg/ml. Specific activities and radiochemical purities were determined for each formulation and the actual radiochemical dose given was determined by measuring the residual ^{14}C-cyhalothrin from each dose. Rats were placed in glass metabolism cages and urine and faeces were collected every 24 hours for up to seven days after dosing. At that time, the animals were killed by CO_2 and selected tissues were removed for measurement of residual radioactivity. In the studies where rats were dosed orally at 1mg/kg, the expired air from two males and two females was monitored for CO_2 for the first 48 hours after dosing.

Excretion Studies in Bile Duct Cannulated Rats

Two studies were conducted with bile duct cannulated rats. In the first study, four male and four female cannulated rats were orally dosed with 1 mg/kg ^{14}CHCN. The total bile produced was collected every 12 hours for up to 48 hours and then to 72 and 96 hours after

dosing. Urine and feces were also collected daily for up to 96 hours. In the second study, four pairs of male rats were cannulated such that for each pair, the bile outflow of one rat was introduced into the duodenum of the second rat via the existing bile duct outlet. Each bile recipient rat was given a single oral dose of 1 mg/kg ^{14}CHCN and the bile, urine and feces were collected as in the previous study.

Blood Collection of Radiolabelled Components

Blood Concentrations of Total Radioactivity

Six male and six female rats per dose were given single doses of ^{14}CHCN (1 and 25 mg/kg orally and 1 mg/kg s.c.) and 1 mg/kg ^{14}C-cyclopropyl. Blood samples were taken from the tail vein of each rat into heparinised tubes at the following times: predose and 15 or 30 minutes, 1, 2, 4, 7, 12, 20, 24, 36 and 48 hours after dosing. The whole blood was analysed for total ^{14}C content.

Blood Concentrations of Total Radioactivity and Unchanged ^{14}C-Cyhalothrin

Twelve male and twelve female rats were dosed orally with either 1 or 25 mg/kg ^{14}CHCN. Three rats of each sex were killed at 2, 7, 24 and 36 hours after dosing, and total blood was collected by cardiac puncture. Each blood sample was analysed for total ^{14}C concentration, plasma ^{14}C concentration and total cyhalothrin concentration.

Analysis of Sample Radioactivity

The radioactivity in prepared samples of whole urine, bile, plasma, feces and tissues collected from the preceding experiments was measured with an Intertechnique SL 30 or SL 4000 liquid scintillation counter. The concentrations of cyhalothrin in whole blood were determined by solvent extraction followed by gas-liquid chromatography. The radiochemical purity of the ^{14}C-cyhalothrin dose formulations and the patterns of radioactive metabolites in the urine, bile and methanol extracts of feces were determined by thin-layer chromatography. Radioactive areas on the developed chromatograms were located by autoradiography and quantitated, either by means of a chromatogram scanner or by a scintillation counter (using scraped segments from each plate). Selected urine samples were treated with either beta-D-glucuronidase or aryl sulphatase. These were then analysed along with control samples by thin layer chromatography.

Results

Excretion Studies With ^{14}CHCN

After oral administration of single doses of ^{14}CHCN to male and female rats at 1 and 25 mg/kg, most of the radioactive dose was rapidly eliminated from the body via the urine and feces. Total urinary (including cage washes) and fecal excretion expressed as the percent of the administered dose were as follows: 1 mg/kg - females excreted 41.5±9.4% in the urine and 46.5±7.5% in the feces

and males excreted 30.0±12.4% in the urine and 61.4%±14.4% in the feces; 25 mg/kg - females, 40.9±9.4% in the urine and 40.2±7.6% in the feces, and males, 40.3±10.7% in the urine and 49.7±14.8% in the feces. The majority of the radioactivity excreted by both routes was recovered in the 0-24 hour samples. There was no detectable excretion of $^{14}CO_2$ in exhaled air. The residues of ^{14}CHCN remaining in the carcasses (after removal of some tissues) seven days after dosing were approximately two and three percent of the dose for males and females respectively at both dose levels.

Following subcutaneous administration of one dose of 1 mg/kg ^{14}CHCN to male and female rats, total recovery of ^{14}C from excreta throughout seven days was 22.2±20.5% in males and 24.7±17.1% in females. Urinary excretion was the predominant route of elimination with 16.4±15.8% and 17.6±12.3% in males and females respectively. Most of the radioactivity remained in the carcasses (less tissues) (58.1±28.7% and 58.8±19.1% for males and females respectively). Measurements of the residual radioactivity in twelve tissues removed from animals seven days after dosing with either 1 or 25 mg/kg ^{14}CHCN indicated that the tissue concentrations were very low with the exception of fat. It should be noted here that although it is not entirely clear, it appears that the tissues for 1 mg/kg ^{14}CHCN and for 25 mg/kg ^{14}C-cyclopropyl were stored for approximately three years at -20°C at which time the ^{14}C residues analysis was conducted.

Excretion Studies With Bile Duct Cannulated Rats

Studies with bile duct cannulated rats dosed orally with ^{14}CHCN showed that there was some excretion of radioactivity via the bile. However, with these rats, the total amounts of radioactivity excreted in the urine and bile were significantly less than the amounts excreted by intact rats administered the same dose. When replacement bile was given to bile duct cannulated male rats, the amounts of radioactivity excreted in both the urine and the bile doubled, suggesting that cyhalothrin is absorbed with the fats of the oil formulation used and that the presence of bile greatly enhances its absorption when administered orally.

Excretion Studies With ^{14}C-Cyclopropyl

As with ^{14}CHCN, most of the administered single oral doses of ^{14}C-cyclopropyl to male and female rats were excreted in the urine and the feces; however, at a much slower rate. Less amounts were excreted in the urine than with ^{14}CHCN, but comparable amounts were excreted in the feces. Again, no detected $^{14}CO_2$ was excreted in exhaled air, only 1-3% of the dose was detected in the carcasses of the rats after seven days, and fat was the tissue with the highest amounts of residual radioactivity after seven days. Residues in fat were similar with both forms of ^{14}C-cyhalothrin indicating that the fat residues may be due to unchanged cyhalothrin.

Blood Concentrations of Radiolabelled Components

Following single oral doses of either 1 mg/kg or 25 mg/kg ^{14}CHCN, the blood concentrations of ^{14}C rose and peaked between four

and seven hours after dose administration. There was no difference between males and females. The mean blood ^{14}C profile at 1 mg/kg showed a two exponential decline with a terminal phase $t_{1/2}$ of about 11 hours. The profile at 25 mg/kg showed a single exponential decline with a $t_{1/2}$ of 11 hours.

Rats dosed subcutaneously with 1 mg/kg ^{14}CHCN showed very low blood concentrations with wide inter-animal variation. In males the mean peak concentration was achieved in approximately 20 hours and in females it was approximately four hours.

Blood Concentrations of Total Radioactivity and Unchanged ^{14}C-Cyhalothrin

In this study the concentrations of total radioactivity and unchanged cyhalothrin in the blood were measured in rats at various times following oral administration of either 1 mg/kg or 25 mg/kg ^{14}CHCN. The data show that the majority of the ^{14}C-labelled material in the blood does not correspond to the presence of intact cyhalothrin.

Chromatographic Analysis of Radioactive Material Excreted by Rats

Thin layer chromatography of ^{14}CHCN and its metabolites in both urine and bile indicated extensive metabolism to polar metabolites. No uncharged ^{14}CHCN was found in either urine or bile. The radioactive material which was quantitatively extracted from feces samples consisted of mainly uncharged compound together with small amounts of more polar metabolites. Treatment of the urine samples with beta-glucuronidase or aryl sulphatase produced no change in the chromatography patterns.

Chromatography of ^{14}C-cyclopropyl and its metabolites in the urine also showed that there was no uncharged compound in the urine. The metabolite patterns, however, were completely different from those derived from the ^{14}CHCN sample.

Discussion

The data from this study suggest that cyhalothrin is not completely absorbed when administered orally to rats and that when it is absorbed, it is extensively metabolised. Following oral dosing, there was a high proportion of uncharged compound excreted in the feces and there was an absence of intact compound in the bile. Urinary excretion was the major route of excretion following subcutaneous administration. In this case the ratio of urinary excretion to fecal excretion was approximately 2.5:1. Therefore, since up to 40% of an oral dose was excreted in the urine, an estimate of approximately 55% absorption was calculated for cyhalothrin. A small proportion of cyhalothrin was retained in the animals seven days after oral dosing, mostly in the fat. Over 50% of the dose was retained in the carcass seven days after subcutaneous dosing. This may have been due to retention in the subcutaneous fat. Blood concentrations in the subcutaneous studies were also considerably lower.

The metabolite patterns from cyhalothrin labelled in two separate positions were completely different, suggesting that metabolism includes cleavage of the ester to yield the corresponding cyclopropylcarboxylic acid and phenoxybenzyl derivatives.

005100

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Secondary Reviewer: Edwin Budd
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DATA EVALUATION REPORT

STUDY TYPE: Metabolism 85-1

ACCESSION NUMBER: 073217

TEST MATERIAL: Cyhalothrin

SYNONYMS: ^{14}C-ICI 146,814: (R,S)Alpha-cyano-3-phenoxybenzyl(+)-cis-3-(2-2-chloro-3,3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate; ^{14}C-benzyl-, ^{14}C-cyclopropyl-ICI 146,814; ^{14}CHON; batches IR2 (19.52 microCi/mg) and 2R3 (10.49 microCi/mg)

STUDY NUMBER(S): ICI No. 146814 MMR 002/03

REPORT NUMBER: Protocol Number ICI 146814 MPM 01

SPONSOR: Imperial Chemical Industries PLC (ICI)

TESTING FACILITY: ICI Pharmaceuticals Division, Safety of Medicines Dept.

TITLE OF REPORT: Cyhalothrin: The Metabolism and Disposition of ^{14}C-ICI 146,814 in Rats: Part III - Studies to Determine Radioactive Residues in the Rat Following 14 Days Repeated Oral Administration

AUTHOR(S): M. P. Harrison

REPORT ISSUED: September 13, 1984

IDENTIFYING VOLUME: Volume II, Book 15 of 16 (Tab Reference 19C)

CONCLUSION: This study, in combination with the other two metabolism studies on the rat, is considered to be CORE GUIDELINE (see comments on Rat Metabolism Study: Parts I and II.

Classification: CORE GUIDELINE

MATERIALS AND METHODS:

Chemical

As stated in the previous study, two radiolabelled forms of cyhalothrin were used for this study, ^{14}CHON and ^{14}C-cyclopropyl (see previous review). Both preparations were greater than 99.8% radiochemically pure with less than 0.18% of the trans isomers. Solutions were prepared by dissolving the compound in corn oil to give a solution of nominal concentration 0.5 mg/ml.

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Animals

Twelve male and twelve female Alderly Park strain albino rats weighing between 200 and 250 g were used for the study. Six animals of each sex were assigned per treatment group.

Study Design

The first group was treated with one oral dose of 0.5 ml ^{14}CHCN per day by gavage for 14 days and the second group was treated with the same amount of ^{14}C-cyclopropyl. The total dose received by each rat over 14 days was determined by measuring the residual radioactive material in each dose vial and syringe and subtracting this value from the starting amount.

Urine and feces were collected separately every 24 hours at intervals of up to seven days after the final dose until the animals were killed. Two animals of each sex were killed at 48 hours and 120 hours after the last daily dose and tissues were removed for measurement of residual radioactivity. The remaining animals were killed seven days after the final dose and tissues were removed as before. The following tissues were removed and stored at -20°C prior to analysis: heart, brain, lungs, spleen, kidneys, gonads, brown fat, white fat, muscle, bone, blood and residual carcass. Urine, feces and tissues were prepared for liquid scintillation counting. The proportions of radioactive material in rat fat samples corresponding to cyhalothrin were determined by solvent extraction followed by HPLC using cyhalothrin standards.

Results

Excretion of Radioactive Material by Rats After Administration of ^{14}C-Cyhalothrin at 1 mg/kg

Over 90% of the cumulative total dose was eliminated in the urine and feces within seven days of the final dose. The overall recovery of radioactive dose for each group was 96%± 1. Excretion by each route apparently reached constant rate after the first or second dose. The total elimination was rapid and very similar in each group. The overall excretion rate expressed as a percent of the average daily dose was 94%/day for males and 93%/day for females given ^{14}CHCN, and 91%/day for males and 92%/day for females given the ^{14}C-cyclopropyl label. There were significant differences in the relative proportions of dose excreted in urine and feces by rats given the two labelled forms of cyhalothrin and also between males and females given the same labelled form. With ^{14}CHCN, male rats eliminated equal amounts of radioactivity whereas females excreted a greater proportion in the urine. With ^{14}C-cyclopropyl, males excreted a much smaller amount of the dose in urine (30%) but females excreted a similar amount as with ^{14}CHCN.

Tissue Residues of Radioactive Material

Residual radioactivity was present in all tissues examined. Fatty tissue showed accumulation of material (white fat up to 88 times the blood level) although lungs, liver, kidney and gonads all had

concentrations 2 to 7 times the blood level (0.048 micrograms/ml). The radioactivity level in the latter tissues depleted considerably seven days post dosing period, although still higher than blood levels. White fat levels did not significantly decrease after seven days. White fat samples were analyzed by extraction and HPLC. With the exception of one animal, most of the radioactivity detected in the tissue was due to uncharged cyhalothrin. The exception was excluded because of poor recovery in the solvent extract.

Discussion

The distribution patterns and excretion rates of radioactively labelled cyhalothrin in rats following administration of multiple oral doses over a period of 14 days were very similar to those found in single dose studies. A large proportion of an oral dose was rapidly eliminated from the body. In the multiple dose study, excretion in urine was slightly higher than in the single dose studies, which may have been due to differences in absorption in normally fed animals as opposed to fasted animals. The data indicate that accumulation of unchanged cyhalothrin in the fat will occur on chronic administration. Otherwise, the compound is rapidly metabolised and excreted.

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0051

DATA EVALUATION REPORT

STUDY TYPE: Metabolism 85-1

ACCESSION NUMBER: 073217

TEST MATERIAL: Cyhalothrin

SYNONYMS: (R,S)alpha-cyano-3-phenoxybenzyl (+)-cis-3-(2-chloro-3,3,3-trifluoropropyl-enyl)-2,2-dimethylcyclopropane carboxylate; ^{14}CHCN; ^{14}C-cyclopropyl; ^{14}C ICI 146,814; ^{14}C-benzyl-ICI 146,814 ^{14}C-Cyclopropyl-ICI 146,814

STUDY NUMBER(S): Not given

REPORT NUMBER: Protocol Number MPH 01

SPONSOR: Imperial Chemical Industries PLC (ICI)

TESTING FACILITY: ICI Pharmaceuticals Division, Safety of Medicines Dept.

TITLE OF REPORT: Cyhalothrin: The Metabolism and Distribution of ICI 146,8 in the Rat: Part IV - Isolation and Identification of the Major Urinary Metabolites Derived From ^{14}C-Benzyl- or ^{14}C-Cyclopropyl-ICI 146,814 Following Oral Administration

AUTHOR(S): M. P. Harrison

REPORT ISSUED: March 23, 1983

IDENTIFYING VOLUME: Volume II, Book 15 of 16 (Tab Reference 19C)

CONCLUSION: This study, in combination with the other two metabolism studies on the rat, is considered to be CORE GUIDELINE (see comments on rat metabolism study: Parts I and II).

Classification: CORE GUIDELINE

MATERIALS AND METHODS:

Chemical

As in the previous two studies, two radiolabelled forms of cyhalothrin were synthesised and used for this study (^{14}CHCN and ^{14}C-cyclopropyl, see previous reviews). Both preparations were greater than 99.7% pure.

Animals

Male and female Alderly Park rats (Alpk/Ap) were selected for the study.

Study Design

Animals were housed in metabolism cages throughout the study. For the study with ^{14}CHCN, six male and six female rats were administered approximately 12.5 mg/kg/day ^{14}CHCN orally for a period of eight days such that each animal received a total of 25 mg of the chemical. Urine and feces were collected every 24 hours up to three days after the last dose. Total urine samples were pooled for each sex, millipore filtered and acidified to pH 1.5 prior to analysis.

For the studies with ^{14}C-cyclopropyl, pooled urine samples from the previous study (where animals received 14 consecutive daily doses of 1 mg/kg ^{14}C-cyclopropyl) were combined with the residual material from the ^{14}CHCN label metabolism study mentioned in the previous paragraph. It was assumed that the residual material after removal of the ^{14}CHCN-labelled components would contain non-radioactive metabolites of which the cyclopropyl moiety would also be present.

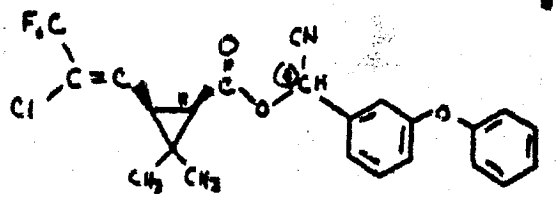
Thin layer chromatography (tlc) was conducted on the prepared urine samples using two solvent systems: chloroform : acetic acid 95:5 (v/v) and ethyl acetate : formic acid : water 70:4:4 (v/v). Radioactive areas on developed tlc plates were detected and quantified using a Berthold LB2722 Radiochromatogram Scanner.

^{14}C-components in urine were also analyzed and purified by reverse phase HPLC using either a Pye Unicam system incorporating an LC3 X P pump, LC X P controller, Altex U.V. detector (254 nm), Berthold LB503 Radioactivity Monitor and Commodore PET computer, or a Dupont 8800 system with a Berthold LB504 Radioactivity Monitor. The solvent systems were various compositions based on acetonitrile:water (+0.1% acetic acid). Purified samples were analyzed via mass spectrometry (electron impact mass spectra and fast atom bombardment mass spectra) and nuclear magnetic resonance spectroscopy.

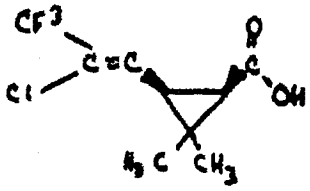
RESULTS:

The analyses conducted above showed that cyhalothrin is extensively metabolized in the rat prior to excretion. The following metabolites were identified in the urine:

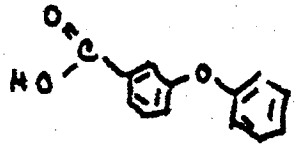
* ⓡ Alternative labels



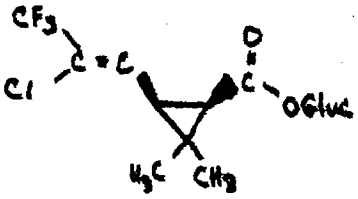
Cyhalothrin (Parent compound - not present in urine)



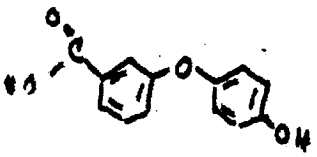
Cyclopropyl carboxylic acid



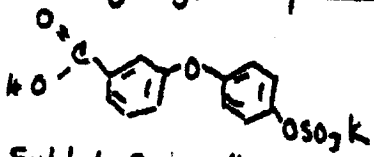
3-Phenoxybenzoic Acid



Glucuronide Conjugate



3-(4'-hydroxyphenoxy)benzoic Acid



Sulphate Conjugation

DISCUSSION: (see previous metabolism studies on the rat).

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Budd 7/25/85

DATA EVALUATION REPORT

STUDY TYPE: Metabolism Study 85-1

ACCESSION NUMBER: 073217

TEST MATERIAL: Cyhalothrin

SYNONYMS: (R,S)alpha-cyano-3-phenoxybenzyl (±)-cis-3-(Z-2-chloro-3,3,3-trifluoropropyl-enyl)-2,2-dimethylcyclopropane carboxylate; ICI 146,814; ^{14}CHCN; ^{14}C-cyclopropyl- and ^{14}C-benzyl-ICI; benzyl: batch 1R4; cyclopropyl: batches 2R3, 2R2 and 2R4

STUDY NUMBER(S): ICI Study Number 146814 KMD 005

REPORT NUMBER: Quality Assurance Unit (ICI) RA841740

SPONSOR: Imperial Chemical Industries PLC

TESTING FACILITY: ICI Pharmaceuticals Division, Safety of Medicines Dept.

TITLE OF REPORT: Cyhalothrin (ICI): The Disposition and Metabolism of (^{14})-ICI 146,814 in The Dog

AUTHOR(S): A. G. Fowkes, M. P. Harrison, T. R. Marten

REPORT ISSUED: September 17, 1984

IDENTIFYING VOLUME: Volume 11, Book 15 of 16 (Tab Reference 20C)

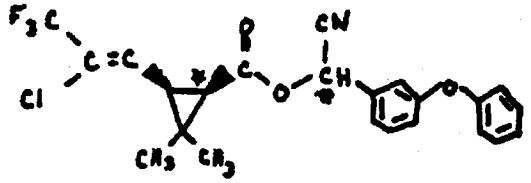
CONCLUSION: This study is classified as CORE MINIMUM because distribution studies were not conducted and a repeated dose absorption, metabolism, distribution and excretion study was not done.

Classification: CORE MINIMUM

MATERIALS AND METHODS:

Chemical Formulations

Two radiolabelled forms of cyhalothrin were used for these studies. The positions of radiolabelling are shown in the following figure:



The abbreviations ^{14}C-benzyl and ^{14}C-cyclopropyl are used to refer to the compound labelled at positions marked $ or * respectively, as shown above. The labelled forms were synthesized by the Radiochemical Unit of the Drug Metabolism Section at ICI Pharmaceuticals Division. For the oral formulations, the radiolabelled compounds were diluted with hexane and corn oil and then the hexane was removed under N_2 at 37°C. For the intravenous studies, the hexane was removed first and the material was re-dissolved in absolute ethanol and diluted with saline. For the individual doses, the radiolabelled ICI 146,814 was diluted with non-labelled cyhalothrin from batch ADM 46156/80 (greater than 99% pure cis 2). The radiochemical dose to each animal was approximately 100 microCi for the oral studies and 50 microCi for the intravenous studies.

Animals

The same three male and three female Alderly Park Beagle dogs were used for all the single dose excretion studies. The dogs weighed approximately 15 kg each.

Single Dose Excretion Studies

The oral studies were conducted at dose levels of 1 and 10 mg/kg and the intravenous studies were conducted at a dose level of 0.1 mg/kg. Since the same animals were used for all of the studies, three weeks were allowed to elapse between each dosing. The studies were conducted in the following order: 1 mg/kg oral benzyl label, 1 mg/kg oral cyclopropyl label, 10 mg/kg oral benzyl label, 10 mg/kg oral cyclopropyl label, 0.1 mg/kg i.v. cyclopropyl label and 0.1 mg/kg i.v. benzyl label. The specific activities of each formulation were as follows: 1 mg/kg benzyl (7.78 microCi/mg), 10 mg/kg benzyl (0.64 microCi/mg), 0.1 mg/kg benzyl (30.5 microCi/mg), 1 mg/kg cyclopropyl (7.07 microCi/mg for males and 6.28 microCi/mg for females), 10 mg/kg cyclopropyl (0.69 microCi/mg) and 0.1 mg/kg cyclopropyl (30.8 microCi/mg). The animals were housed in individual metabolism cages. Urine, feces and cage washes were collected at 24-hour intervals from the time of dosing up to seven days. For the oral 10 mg/kg cyclopropyl label study, urine was collected at 0-8 and 8-24 hours in addition to the 24-hour intervals. Blood samples were collected at pre-dose, 1, 2, 4, 6, 12, 24 and

every 24 hours thereafter for up to 168 hours post dosing. For the intravenous studies, additional samples were taken at 0.5 and 8 hours. Samples were stored at -20°C until analyzed.

Determination of Total Radioactivity in Urine, Feces, Cage Washes, Plasma and Whole Blood

Samples were prepared for liquid scintillation counting. Feces and whole blood were prepared by sample oxidation. The CO_2 produced during oxidation was absorbed in 2-methoxyethylamine and mixed with a toluene based scintillant.

Analysis of Sample Radioactivity

Urine samples were either treated with various enzyme preparations; acidified to pH 1 or basified to greater than pH 10 and heated at 80°C for 30 minutes; or left untreated in pH 5 acetate buffer and analyzed further. The enzyme preparations consisted of combined beta-glucuronidase and sulfatase type H-1 (with and without 1,4-saccharolactone which inhibits beta-glucuronidase activity), sulphatase type V with 1,4-saccharolactone, and beta-glucuronidase type IX. Test incubations were conducted using phenolphthalein glucuronide and p-nitrocatechol as substrates. Feces homogenates were extracted with methanol.

The patterns of radioactivity in the urine and feces samples were analyzed by thin layer chromatography (tlc) using one of the following solvent systems: chloroform:acetic acid (95:5 v/v); ethyl acetate:formic acid (98%):water (70:4:4 v/v); toluene:n-hexane:acetonitrile:chloroform (200:100:2:5 v/v) or toluene:ethanol (2:1 v/v). Radioactive areas were located by autoradiography and scanned.

The ^{14}C-benzyl metabolites were extracted from urine samples from one male and one female dog from the 10 mg/kg oral study using n-hexane (male dog only) and ethyl acetate (both dogs) as extraction solvents. The metabolites were then analyzed by tlc using the second solvent system in the list above. Radioactive areas were excised and further purified by preparative tlc using the first solvent system followed by a third tlc in either ethyl acetate:methanol:water (13:2:1 v/v) or chloroform (saturated with 90% formic acid):diethyl ether (10:3 v/v). Samples were then further analyzed by mass spectrometry.

The ^{14}C-cyclopropyl metabolites were extracted from male urine from the 10 mg/kg oral study using ethyl acetate as the extraction solvent. The samples were analyzed by chromatographing and re-chromatographing with tlc using the second solvent system. Samples selected for further clean up were first chromatographed in chloroform:methanol:acetic acid (10:5:2 v/v) followed by preparative tlc in ethyl acetate:methanol:water (13:2:1 v/v) and rechromatographed again in the second solvent system. For the mass spectrometry, metabolites were compared with known reference materials where possible.

RESULTS:

Disposition of ^{14}C-Benzyl-ICI in the Dog

1 Mg/Kg Oral Dose

The diluted ^{14}C-labelled compound used was greater than 97% pure ^{14}C-ICI. Most of the radioactivity was excreted during the first 48 hours after dosing, mainly via the feces (in both males and females). The mean values at 48 hours were: 75.6% of total dose excreted (excluding cage washes), 24.8% in urine and 50.8% in feces. After 7 days the total excretion of radioactivity including cage washes amounted to 86.0 ± 4.5% (54.2 ± 3.9% in feces and 29.7 ± 7.3% in urine).

The radioactivity in whole blood was found to be attributable to the radioactivity in plasma. Plasma concentrations of radioactivity rose rapidly and peaked between 2 and 12 hours post dose. Three dogs gave secondary peaks at 12 hours while others showed a delayed fall in levels. The half-life of the decline in plasma levels was calculated to be 28 hours.

10 Mg/Kg Oral Dose

Excretion rates were similar to the 1 mg/kg group. 68.8% of the radioactivity was excreted in the first 48 hours. Mean plasma levels peaked at 2 hours post dosing and again at 12 hours post dosing. The half-life of the decline in plasma levels was calculated to be 32 hours.

0.1 Mg/Kg Intravenous Dose

The diluted ^{14}C-labelled compound used was greater than 96% pure ^{14}C-ICI. Excretion patterns were different from those in the oral studies in that significant amounts of radioactivity were excreted over the first three days (as opposed to the first 48 hours) and that radioactivity was more evenly distributed between urine and feces in both males and females. The mean values at 72 hours for males and females combined were: 32.7% of the total dose in urine and 37.1% of the total dose in the feces. Approximately 83% of the dose was recovered in urine, feces and cage washes after 7 days.

Plasma concentrations fell rapidly until 4 hours after dosing and then rose to a peak at 12 hours. Thereafter levels fell again with a half-life of 33.6 hours.

Analysis of Radioactivity in the Urine

TLC analysis of 0-24 hour urine samples indicate that ^{14}C-benzyl-ICI is extensively metabolized in the dog. No parent compound was found in the urine. The following metabolites were identified by TLC and mass spectrometry: 3-phenoxybenzoic acid (3-PBA) and glucuronic acid conjugate, 3-(4-hydroxyphenoxy)benzoic acid and sulphate, N-(3-phenoxybenzoyl)-glycine and two unknowns.

Analysis of Radioactivity in Feces

TLC of methanol extracts of feces samples indicated that for both dose levels 1 mg/kg and 10 mg/kg (oral), the main component excreted within the first 24 hours was unchanged cyhalothrin (74.4% of applied radioactivity for a male dog at 1.0 mg/kg and 93% for a female dog at 10 mg/kg). The sample from the male dog also contained three other components, two bands with similar R_F's to 3-PBA, one which was more polar, and one which was less polar than 3-PBA and may have been a metabolite of the intact ester. The female dog also had a component with a similar R_F to 3-PBA. Fecal samples taken from a female dog between 24 and 48 hours post dosing with 1.0 mg/kg contained only 8.5% unchanged compound and 5 or 6 other components. Samples taken from another female dog between 0 and 24 hours post dosing with 0.1 mg/kg ^{14}C-benzyl-ICI intravenously showed a pattern very similar to the 24-48 hour samples from the 1.0 mg/kg dosed dog. Only 1.5% of the radioactivity present was from unchanged cyhalothrin. Five or six other components were present in similar amounts as the 1.0 mg/kg dog, one of which had a similar R_F to 3-PBA (39.9% of the dose).

Disposition of ^{14}C-Cyclopropyl-ICI in the Dog

1 Mg/kg Oral Dose

The diluted ^{14}C-labelled compound used was greater than 98% pure ^{14}C-ICI. Excretion patterns were similar to those with ^{14}C-benzyl-ICI in that most of the dose was excreted during the first 48 hours, mainly via the feces. There were no significant differences between males and females.

Again, the radioactivity in whole blood was found to be attributable to the radioactivity in plasma. Concentrations in plasma peaked at four hours post dose and then fell, rapidly at first and then more slowly.

10 Mg/kg Oral Dose

Oral administration at this dose had an emetic effect on several dogs, which were subsequently excluded from the data. Two of the dogs lost greater than 10% of the dosed radioactivity. There was some difficulty in obtaining fecal samples; however, excretion of radioactivity still appeared to occur predominantly within the first 24 hours after dosing. In females, 2/3 dogs failed to produce feces, which delayed excretion somewhat. Concentrations in plasma peaked at 12 hours and subsequently declined.

0.1 Mg/kg Intravenous Dose

Radioactivity was excreted rapidly via both urine and feces in approximately equal amounts. The mean total recovery over 7 days was 81.9% with 40.0% in the urine and 38.7% in the feces. The balance was in the cage wash. Concentrations in plasma fell rapidly after dosing.

Analysis of Radioactivity in the Urine

Analysis by TLC and mass spectrometry indicate that this part of the molecule is extensively metabolized. At least twelve metabolites were identified in the urine, some present in both the free form and the conjugated form. There was a variation in the pattern of the metabolites which was dependent upon dose level, route or sex.

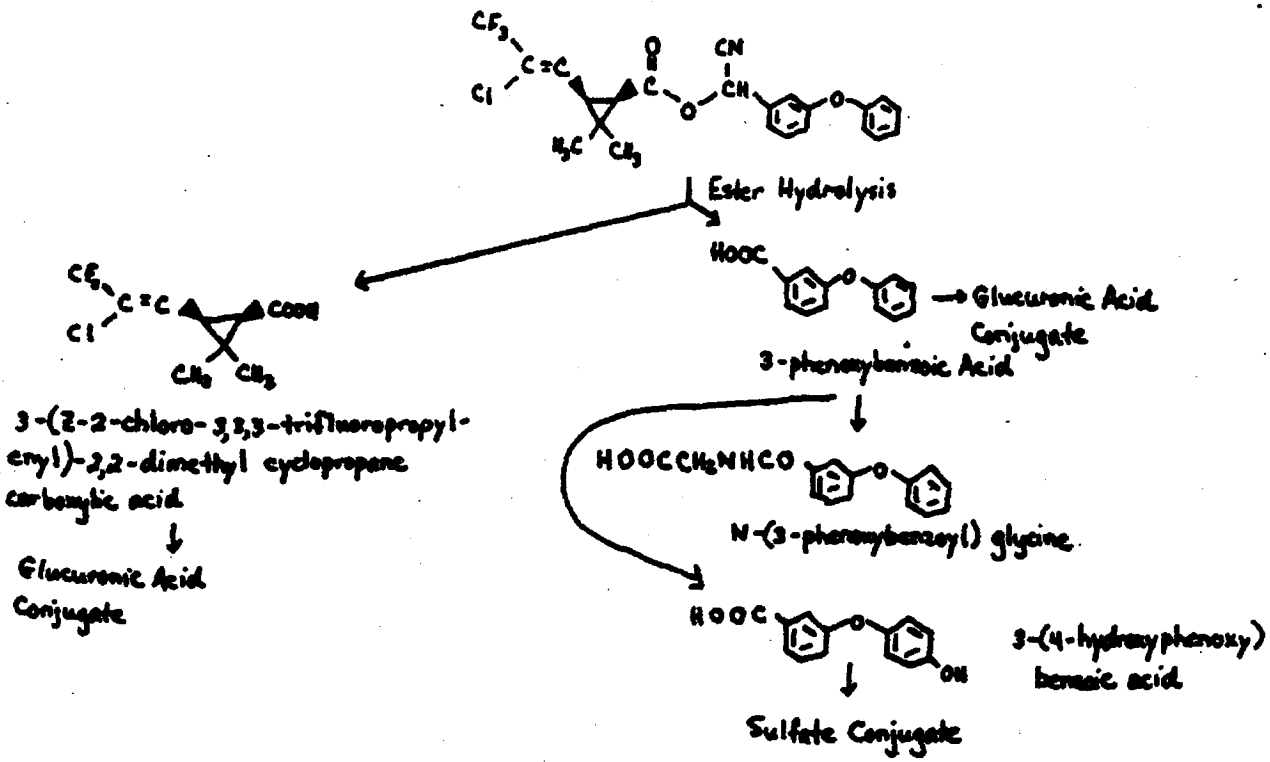
Analysis of Radioactivity in the Feces

At both dose levels 1 mg/kg (oral) and 10 mg/kg (oral), the major component was unchanged cyhalothrin which was mostly excreted during the first 24 hours. Between 24 and 48 hours, 3-5 other components were observed as well, two chromatographing at R_F 0.56 and two more polar components chromatographing at R_F 0.25 and at the origin. Samples were not taken for the 10 mg/kg dose level beyond 24 hours. When ^{14}C-cyclopropyl-ICI was administered intravenously at a dose level of 0.1 mg/kg, the pattern was similar to the pattern observed with 1.0 mg/kg (orally) between 24 and 48 hours. Even less unchanged cyhalothrin was observed in the feces when the compound was administered intravenously (1.4% of the administered dose within the first 24 hours).

DISCUSSION:

Using the urinary excretion data from the intravenous studies and from the lower dose oral studies, the authors concluded that for the ^{14}C-benzyl label the absorption was 80% and for the ^{14}C-cyclopropyl label the absorption was 48%. The high dose oral studies could not be used for this purpose because of fecal contamination of the urine. The authors stated that the discrepancy in absorption rates was probably due to inter-animal variation. This plausible, but is not definitively proven in the study.

The metabolite patterns from each of the two radiolabelled cyhalothrin compounds were quite different from each other indicating extensive cleavage of the ester bond. Urinary metabolites from the ^{14}C-benzyl studies are listed in the results section of this review. There were up to seven metabolites isolated. Twelve metabolites were isolated from the ^{14}C-isopropyl studies. In the feces, a large proportion of the radioactivity was due to unchanged cyhalothrin. One metabolite was found to be common to both labelled studies. Because of its properties, it is thought to be a metabolite of the intact ester. The following figure depicts the identified metabolites of cyhalothrin in the dog:



Excretion in all studies was rapid in both urine and feces, nearly all of it within 48 hours. The difference between the amount of unchanged compound found in the feces in the oral studies versus the intravenous studies was so pronounced that it appears that absorption of the compound is incomplete.

The rat studies indicate that some of the compound is retained in the fat and released slowly. If this is the case with the dog study, then it would partly explain the lack of complete recovery of radioactivity from the initial dose.

lewed by: Pamela Hurley
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Secondary Reviewer: Edwin Budd
Section 2 , Tox. Branch (TS-769C)

005316

DATA EVALUATION REPORT

STUDY TYPE: Metabolism (85-1) - rat TOX. CHEM. NO.: 271F

ACCESSION NUMBER: 073981

TEST MATERIAL: (R,S) alpha-cyano-3-phenoxybenzyl (1R,S)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate and the radiolabelled ^{14}C version

SYNONYMS: Cyhalothrin, PP563, (Grenade is the formulation)

STUDY NUMBER(S): UR0169

REPORT NUMBER: CTL/P/1014

SPONSOR: ICI PLC Plant Protection Division, Bracknell, Berks, UK

TESTING FACILITY: ICI PLC Cntrl. Tox. Lab, Alderly Park, Macclestield, UK

TITLE OF REPORT: Cyhalothrin: Bioaccumulation in the Rat

AUTHOR(S): Prout MS

REPORT ISSUED: July 31, 1984

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab 26C

CONCLUSION: Cyhalothrin is taken up slowly by fat and released slowly. It is rapidly released by the blood, kidneys and liver. The data indicate that the rate of metabolism of both enantiomer pairs of cyhalothrin is likely to be identical, which means that the rate of metabolism of PP321 is likely to be identical to cyhalothrin.

Classification: This study, in combination with previous metabolism and distribution studies conducted on cyhalothrin (previous submission), is classified as Core Guideline.

MATERIALS AND METHODS:

Chemical:

Cyhalothrin and ^{14}C-cyhalothrin were obtained from ICI PLC, Plant Protection Division, Bracknell, Berks, UK. The purity of the non-radiolabelled chemical was 92.2%. The radiochemical purity of the radiolabelled chemical was 98.6%. The position of the radiolabel was on the cyclopropane ring.

Animals:

Adult male Alpk/AP strain rats were obtained from the Alderly Park animal breeding unit.

Protocol:

The dosing solution was prepared by mixing unlabelled cyhalothrin, radiolabelled cyhalothrin and corn oil to achieve a final concentration of 0.5 mg/ml cyhalothrin in corn oil (approximately 0.05 MBq/mg cyhalothrin). Animals were dosed once daily for up to 119 consecutive days (bodyweight dependent doses; controls received corn oil alone). Groups of 3 treated and 1 control were sacrificed after every 7 doses (24 hours following last dose), for up to 77 dosages and then after 91, 105 and 119 doses. Upon sacrifice, samples of blood and fat and the liver and kidneys were taken for radioactivity analysis. The liver and kidneys were weighed. The samples were combusted in a Packard Tricarb model B306 sample oxidizer and analyzed for radioactivity. Oxidation efficiencies of <92% were rejected. In addition, fat samples were extracted with hexane and dimethyl formamide, separated by HPLC and counted for radioactivity.

RESULTS:

During the dosing period, the levels of radioactivity in the blood remained between 0.10 and 0.59 micrograms/g blood, average peaking at 0.2 micrograms/g blood. After an initial rise, the levels of radioactivity in liver and kidney appeared to plateau at 2.5 and 1.2 micrograms/g respectively after 70 days of dosing. The levels of radioactivity in these three tissues declined rapidly upon cessation of dosing (levels in kidney and blood barely detectable after 5 weeks and levels in liver declining rapidly at first and then elimination parallelling that of fat). Levels in fat increased with time to a level of approximately 9 micrograms/g at 119 days. Upon cessation of dosing, these levels declined by a first order process (typical exponential decline with time). Separation of the fat extracts by HPLC gave 2 main peaks, corresponding to the 2 enantiomer pairs of cyhalothrin. The ratio of the pairs present in fat was the same as in the dosing solutions. The half-life of cyhalothrin in fat was calculated to be 30.5 days.

DISCUSSION:

Cyhalothrin was taken up slowly in the fat and released slowly. This was not the case in the other tissues. It was eliminated fairly rapidly. In the case of the liver, the small amounts remaining were probably due to amounts being slowly released from the fat tissue. In addition, the data indicated that the rates of metabolism are likely to be identical for both enantiomeric pairs since their ratio was the same for both the dosing solution and the amounts found in fat. Therefore, the rate of metabolism of PP321, which is one of the two pairs of enantiomers, is likely to be identical to the rate of metabolism of cyhalothrin (PP563).

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005316

DATA EVALUATION REPORT

STUDY TYPE: Metabolism (85-1) - rat

TOX. CHEM. NO.: 271F
725C
725B

ACCESSION NUMBER: 073981

TEST MATERIALS: (R,S) alpha-cyano-3-phenoxybenzyl (1R,S)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate; (R+S) alpha-cyano-3-phenoxybenzyl(1S+R)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate; and (R+S) alpha-cyano-3-phenoxybenzyl (1R+S)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl cyclopropane carboxylate

SYNONYMS: Cyhalothrin, PP563; PP321; R157836 respectively

STUDY NUMBER(S): UR0178

REPORT NUMBER: CTL/P/1214

SPONSOR: ICI PLC, Plant Protection Division

TESTING FACILITY: ICI PLC, Cntrl. Tox. Lab., Alderly Park, Macclesfield, UK

TITLE OF REPORT: PP321: Comparative Absorption Study in the Rat (1 mg/kg)

AUTHOR(S): Prout MS and Howard EF

REPORT ISSUED: March 19, 1985

IDENTIFYING VOLUME: Volume II, Book 2 of 2, Section C, Tab 27C

CONCLUSION: The results indicate that the absorption, distribution, metabolism and excretion patterns of PP321 and cyhalothrin following a single 1 mg/kg dose in the male rat are identical.

Classification: When taken with previously submitted metabolism studies, this study is Core Guideline.

MATERIALS AND METHODS:

Chemical:

All chemicals were obtained from ICI PLC Plant Protection Division.
R157836 was prepared from cyhalothrin by HPLC.
Cyhalothrin: Unlabelled purity 97.4% w/w, CTL ref. # Y00102/034/001.
Labelled chemical prepared by mixing equal proportions of the ^{14}C-PP321 and ^{14}C-R157836.
PP321: Unlabelled purity 99.0% w/w, CTL ref. # Y02537/045/001. Labelled chemical labelled in cyclopropane ring, specific activity 1.95GBq/mmole and radiochemical purity of >98%. CTL ref. # Y02537/044/001.
R157836: Unlabeled purity 93.5%, CTL ref. # Y04369/002/001. Labelled chemical labelled in cyclopropane ring, specific activity 1.97GBq/mmole and radiochemical purity of >98%. CTL ref. # Y04369/044/001.

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Animals:

Twelve male Alpk/AP rats (170-250g) were obtained from the Alderly Park Animal Breeding Unit. They were kept in individual metabolism cages.

Protocol:

Three dosing solutions were prepared such that a dose level of 4 ml/kg bodyweight was equivalent to a nominal dose level of:

Dose 1: 1 mg/kg PP321 + 1MBq/kg ^{14}C-PP321
Dose 2: 1 mg/kg PP321 + 1MBq/kg ^{14}C-PP321 + 1 mg/kg R157836
Dose 3: 1 mg/kg cyhalothrin + 1 MBq/kg ^{14}C-cyhalothrin

Four animals per dose group were given one oral dose (4 ml/kg) of the selected dose. Urine and feces were collected over dry ice at 24 hour periods for 3 days and retained at -20°C for analysis. Cage washings were also retained. Upon sacrifice, samples of blood and fat, and the liver and kidneys were removed and retained at -20°C for analysis. The livers and kidneys were homogenized in water and the fat samples were homogenized without water. Feces were homogenized in methanol. Samples of blood, liver, kidneys, fat and fecal residue were combusted and analyzed for radioactivity. Oxidation efficiencies of <92% were rejected. Samples of urine and cagewash were diluted and counted directly.

Zero to 24 hour urine samples and the 0-24 hour and 24-48 hour samples of fecal extracts were retained from each animal and analyzed on thin layer chromatography (TLC) in the following solvent systems:
chloroform : methanol : acetic acid 18:1:1
butan-1-ol : acetic acid : water 9:2:1
The precise location of radiolabel was confirmed by autoradiography. Two tailed Student's t-tests were used to compare one group with another group.

RESULTS:

The total excretion of radioactivity from the 3 groups was very similar. There were no statistically significant differences in the total urinary or the total fecal excretion of radioactivity between the 3 groups. The authors stated that the very low levels of radioactivity found in the blood were close to the limit of detection, thus the apparent differences in levels of ^{14}C-PP321 and ^{14}C-cyhalothrin was probably spurious. They also stated that the differences in liver concentrations between dose groups II and III disappear when the comparison is made on the basis of percentage dose left in the liver at termination. The mean concentrations of radioactivity in the fat of rats dosed with either ^{14}C-PP321 or ^{14}C-cyhalothrin were nearly identical (0.25 and 0.26 microgram equivalents/g fat respectively. In addition, the residue level of radioactivity in fat of animals in dose group II was not significantly different from either of the other groups.

The methanol extract of the 0-48 hour feces from rats in the 3 groups contained a mean of 65% of the total material excreted via the feces in this period. More than half of the material was unchanged cyhalothrin and the major metabolites present were common to all groups. The methanolic trituration of freeze dried urine (0-24 hours) extracted a mean of 90% of the radioactivity present in the urine from rats in all groups. According to the authors, the major peak of radioactivity when chromatographed by TLC was probably the glucuronide of cyhalothrin acid. No unchanged PP321 or cyhalothrin was excreted in the urine, however, the free cyhalothrin acid was a significant urinary metabolite in all groups accounting for between 3-9% of the material present in the day 1 urine.

DISCUSSION:

The results of this study indicate that the absorption, metabolism, excretion and tissue distribution of ^{14}C-PP321 and ^{14}-cyhalothrin are indistinguishable from one another. These results are compatable with previous studies.