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WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID# 069001. Pyrethrins: Review of a series 83-1 chronic feeding study in dogs.

TOX CHEM No.: 715
PC No.: 069001
Barcode No.: D206439
Submission No.: S471631

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I. CONCLUSION

The series 83-1b chronic feeding study in dogs with pyrethrins (MRID NO.: 414965) was reviewed and determined to be CORE GUIDELINE. No additional series 83-1 chronic feeding study in dogs is required at this time. The study was demonstrated to have a NOEL of 500 ppm (13.7 mg/kg/day) and a LEL of 2500 ppm (66.4 mg/kg/day).

II. Action Requested and Discussion.

SRRD has requested that the series 83-1b chronic feeding study in dogs with pyrethrins (MRID NO.: 414965) be reviewed. The study was reviewed and determined to be CORE GUIDELINE. A copy of the study DER is attached. The Executive Summary for this study is presented in Part III below.

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III. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
<p>83-1. Chronic Feeding-dog IRDC Study No.: 556-007, May 18, 1990</p>	<p>Technica. pyrethrins FEK-99</p>	<p>414965</p>	<p>Four groups of 4/sex beagle dogs were dosed as control, 100, 500 and 2500 ppm of pyrethrins in their diets for one year. These dose levels correspond to 2.57, 13.7 or 66.4 for males and 2.8, 14.2 or 74.6 for females mg/kg/day of pyrethrins.</p> <p>The 2500 ppm dose group was associated with male liver weight increase (30%, $p < 0.05$) and females were also increased (25%, not significant). The 2500 ppm dose group also indicated a possible threshold level for anemia (decreased erythrocytes, hematocrit and hemoglobin); alanine aminotransferase was also slightly elevated. The LEL is 2500 ppm (66.4 mg/kg/day) based on liver weight increase. The NOEL is 500 ppm (13.7 mg/kg/day).</p>	<p>GUIDELINE</p>

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1/18/95

DATA EVALUATION REPORT

STUDY TYPE: 83-1. Nonrodent (dogs) chronic feeding.

MRID NO.: 414965

TOX. CHEM. NO.: 715
PC No.: 069001

TEST MATERIAL: Technical pyrethrum, FEK-99.

STUDY NUMBER: 556-007

SPONSOR: Pyrethrin Joint Venture/Chemical Specialties
Manufacturers Association

TESTING FACILITY: IRDC, Mattawan, Michigan

TITLE OF REPORT: "Evaluation of Pyrethrum Extract in a One Year
Chronic Toxicity Study in Dogs".

AUTHOR: Edwin I. Goldenthal

REPORT ISSUED: May 18, 1990

[In life phrase: February 3, 1988 to 52 weeks later.]

EXECUTIVE SUMMARY:

Four groups of 4/sex beagle dogs were dosed as control, 100, 500 and 2500 ppm of pyrethrins in their diets for one year. These dose levels correspond to 2.57, 13.7 or 66.4 for males and 2.8, 14.2 or 74.6 for females mg/kg/day of pyrethrins.

The 2500 ppm dose group was associated with male liver weight increase (30%, $p < 0.05$). Females were also increased (25%, not significant). The 2500 ppm dose group also indicated a possible threshold level for anemia (decreased erythrocytes, hematocrit and hemoglobin); alanine aminotransferase was also slightly elevated. The LEL is 2500 ppm (66.4 mg/kg/day) based on liver weight increase. The NOEL is 500 ppm (13.7 mg/kg/day).

Classification: CORE GUIDELINE. No additional series 83-1 chronic feeding studies in non-rodents are required at this time. No significant study deficiencies were recognized.

Quality Assurance Statement: Provided.

Good Laboratory Practice Statement: Provided.

Statement of No Data Confidentiality Claims. Provided, no claim of data confidentiality on the basis of the scope of FIFRA. Data are considered proprietary to the submitter.

REVIEW

Experimental Constants:

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Test Chemical:

Chemical: Pyrethrum extract
Source: Pyrethrum Task Force and Fairfield American Corporation c/o FMC Corporation
Blend#: FEK-99 (dated 08/05/86)
Composition: 57.574% pyrethrins, balance: plant extract and stabilizers.
Appearance: Amber liquid
Storage: Refrigerated (unspecified temperature) in stainless steel containers.

Analytical Chemistry:

Data on homogeneity analysis indicated that the 100 and 2500 ppm samples had means of 97 ± 21 and 99 ± 2.6 percent for top, middle and bottom samples. Stability analysis indicated no breakdown of the 100 ppm sample but as much as 5% breakdown of the 2500 ppm sample over a period of 14 days at room temperature. The mean concentrations for 16 preparations of the 100, 500 and 2500 ppm diets were 100 ± 5 , 100 ± 4.6 and 99 ± 3.6 percent.

Test System:

Species: Dog/purebred beagle
Supplier: Ridgman Farms, Mt. Horeb, Wisconsin
Age: Approximately 5 months at receipt and 7 months at initiation of dosing.
Weight: Males: 9.4 to 13.9 kg; females 7.2 to 11.9 kg
Randomization: Weight stratified randomization procedure
Housing: Individually
Diet: Certified Canine Diet[®] #5007, Ralston Purina Co.

Basic Experimental Design:

Four groups of 4/sex dogs were dosed as control, 100, 500 or 2500 ppm of pyrethrin based on the actual pyrethrin content for 52 weeks.

These dose levels were selected based on a preliminary 8 week range finding study (IRDC Study No.: 556-006) that was briefly described in the study synopsis. In the preliminary study, dogs were dosed with 600, 1000, 3000 or 6000 ppm of pyrethrin. The 6000 ppm dose level was fatal to two dogs. At 3000 ppm and above, thin appearance, inappetence, ataxia, trembling, oily coat; impaired limb function and shallow breathing were reported; and possible hematological and clinical effects were noted but not described. Absolute liver weight was increased and absolute testis weight appeared to be decreased at 1000 ppm and above. Thus 2500 ppm was determined to be a dose level that would produce pharmacological signs.

Statistics: The study report asserts that the following statistical tests were performed. Tests were two tailed and with $p < 0.05$ and 0.01 used as levels of significance.

Statistical Test	Parameter Investigated
One way analysis of variance and Bartlett's tests for homogeneity of variance followed by Dunnett's multiple comparison tables to determine the significance of difference.	Body weights, food consumption, hematological, biochemical and urological parameters, absolute and relative organ weights.
Non-parametric analysis of Conover and Lamb	Total bilirubin, urine specific gravity, and volume

Specific Methods and Results

1. Survival and clinical signs. The dogs were reported examined twice daily.

All dogs survived the treatment period.

No reactions to treatment were reported except for some initial aversion to the test diets in the two highest test dose groups. This is discussed further under food consumption.

2. Body weight and food consumption and compound consumed. Assessments were made weekly for the first 14 weeks and biweekly thereafter.

The study report asserts that there were no compound related effects on body weight but that there were initial effects on food consumption. Table 1 below illustrates selected body weight, food consumption and total compound intake data.

Table 1. Body weight and gain, food consumption and pyrethrin consumed.

Dose Level	Males				Females			
	Weight Gain ¹	Food Consumed ²	Terminal Weight	Compound Intake ²	Weight Gain	Food Consumed	Terminal Weight	Compound Intake
Control	0.3	341	13.4±4.0	--	0.7	295	10.7±1.7	--
100	1.0	319	13.7±2.0	2.57	0.3	308	11.7±2.4	2.84
500	0.9	297	16.5±1.9 (+48%)	13.7	0.6	265	11.0±1.8	14.1
2500	0.5	288	13.6±1.0	66.4	0.3	260	10.1±0.8	74.4

Data are from Table 2 (body weight) and Table 3 (food consumption) and Table on page 25 of the study report (compound consumed).

1. Weight gain (kg) and food consumption (g/animal/day) over first four weeks of study. The food consumption data entered are the mean value of the gm/animal/day for the first four weeks.

2. In mg/kg of pyrethrin/day.

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There were transient decreases in food consumption and only on week one did the difference reach statistical significance for the male high dose group when it was 30% lower. At week one, female feed consumption was also down 25% but statistical significance was not attained. The high dose group had the lowest mean value for feed consumption rate for the first four weeks.

The 500 ppm dose group males gained more weight than the other groups. This is considered to be an incidental finding not related to treatment because there were no other indications of pyrethrin induced effects to support the weight increase. It must be remembered that there are only 4 dogs/sex/dose limiting the statistical assessment of the data.

3. Ophthalmoscopic Examinations

No effects on the eyes of the dogs were noted at termination as indicated by the assessment of Dr. Lionel Rubin.

Blood and urine samples were taken at 6 months at just prior and at termination. Blood was taken from the jugular vein following a 24 hour fast (no food, no water). Urine was collected on dry ice.

4. Hematology. The following parameters were assessed:

leucocyte count	erythrocyte count
hemoglobin	hematocrit
MCV, MCH, MCHC	platelets
differential WBC	reticulocytes

The study author indicated that increased total leucocyte and segmented neutrophils in females and decreased erythrocytes, hemoglobin and hematocrit in male dogs were possible effects of treatment at the high dose group only. These possible effects are quantitated as follows.

-total leucocyte: (high dose females at week 12 only). The control group was 8.11 ± 2.11 and the high dose group was 17.1 ± 6.23 or 106% higher ($p < 0.05$). The other groups were essentially similar to the control.

-segmented neutrophil: (high dose females at week 12 only). The control group was 4.8 ± 2.43 and the high dose group was 12.9 ± 6.02 or 169% higher ($p < 0.05$). The other groups were essentially similar to the control.

TB-I does not consider these to be an effect of treatment because of the inconsistency over time and large standard deviation in the high dose group.

-erythrocytes, hemoglobin and hematocrit: These parameters were 9 to 14% lower in the high dose group than in the controls at both 6 and 12 month assessments.

It would be expected that if the erythrocyte count decreased the hemoglobin and hematocrit would also decrease. These data indicate that at

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best there is a possible anemic effect at the highest test dose level. Possible anemic effects were noted also in the preliminary range finding study at 3000 and 6000 ppm. Thus, TB-I concludes that the 2500 ppm dose level is a probable threshold level for anemic effects.

5. Clinical chemistry . The following parameters were assessed.

sodium	potassium
chloride	calcium
inorganic phosphorus	alkaline phosphatase
total protein	aspartate amino transferase
alanine amino transferase	creatinine kinase
urea nitrogen	creatinine
total protein	albumin
globulin	cholesterol
glucose	

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The study author asserts that alanine aminotransferase was increased for females at 2500 ppm at both the 6 and 12 month assessment times. Inspection of table 6 of the study report (page 79) indicates that at both 6 (28%, p < 0.05) and at 12 months (39%, p < 0.01) readings for alanine aminotransferase¹ are increased. The study report maintains that although there are increased relative to the control, the values are still within the range of normal values. TB-I concludes that these increases are probably borderline indications of liver toxicity. This will be discussed under liver weight and pathology below.

6. Urinalysis The following parameters were assessed.

volume specific gravity pH

No effects of treatment were reported.

6. Organ weights. The following organs were weighed:

adrenals (2)	brain	heart
kidney (2)	liver	ovary (2)
pituitary	spleen	testis (2)
thyroid/parathyroid complex		

Liver weight was increased as indicated in Table 2 below.

¹Sample readings for alanine aminotransferase were for at 6 months: 29±6.0, 25±0.8, 28±4.5 and 37±4.1 (p < 0.05) U/l for the control, low, mid and high dose groups respectively.

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Table 2. Liver weight

Dose (ppm)	Males			Females		
	Absolute	Relative to BW	Relative to Brain	Absolute	Relative to BW	Relative to Brain
0	293.3 ¹	2.35	3.52	238.0	2.39	3.05
100	306.2 (+4.4%)	2.28	3.73 (+5.0%)	264.6 (+11.1%)	2.47 (+3.3%)	3.61 (+18.4%)
500	348.5 (+18.9%)	2.19	4.35 (+23.6%)	263.0 (+10.5%)	2.45 (+2.5%)	3.42 (+12.1%)
2500	381.9* (+30.2%)	2.92* (+24.2%)	4.78 (+35.8%)	298.2 (+25.3%)	3.06 (+28.0%)	3.94 (+29.5%)

Data are from Table 9 of the study report.

* p < 0.05

1. Data for absolute weight is in grams. Other data are a grams to grams ratio without units.

The study author maintains that the high dose males have significantly increased liver weight*. The above table confirms that conclusion but also strongly suggests that the female high dose group also has increased liver weight.

A possible effect of pyrethrins in the 500 ppm dose male group is obscured because this group had a higher body weight. The liver weight to body weight ratio was not increased but the liver to brain weight was increased indicating a possible effect. In females, the 10% and 12% increases in absolute and liver weight relative to brain weight effect suggests again that the 500 ppm dose group is a threshold for liver weight increase. The limited number of dogs per dose group prevents a more definite conclusion that liver weight is affected at the 500 ppm dose level.

TB-I concurs that the NOEL and LEL for liver weight increases are 500 and 2500 ppm.

The testis weight was indicated in the report for the range finding study as appearing to be decreased at 1000 ppm. In the main study, the testis weight did not show any indication of being affected. For example, the control group was 17.54±2.81 gm and the high dose group was 17.98±3.903 gms. Thus the initial observation in the range finding study was not confirmed in the main study.

7. Pathology. The following tissues were assessed microscopically. The tissues from all dogs on the study were assessed.

adrenal cortex	adrenal medulla	aorta
bone marrow(sternum)	bone(femur)	bone(sternum)
brain	cecum	colon
duodenum	epididymis	esophagus
eye	eye(with optic nerve)	gallbladder
heart	ileum	jejunum
kidney	liver	lung
lymph node(mesenteric, submandibular and tracheobronchial)		
mammary gland	nerve, sciatic	pancreas
parathyroid	pituitary	prostate
rectum	salivary gland	skeletal muscle
skin	skin (ear)	
spinal cord (lumbar, cervical, thoracic)		
spleen	stomach (cardia, fundus, pylorus)	
testis/ovary	thymus	thyroid
trachea	urinary bladder	uterus

The study author asserts that there were no compound related effects noted. The following two organs are discussed individually for the reasons given.

A. Liver. Liver weight was increased in males and probably also in females in the high and possibly mid dose groups. The liver is recognized as a target organ in the rat and the mouse chronic feeding studies. Alanine amino transferase was slightly elevated in this study suggesting liver injury or pathology.

All of the high dose group males were described as being within normal limits. In the mid dose group, two dogs had inflammation but this is not considered sufficient evidence of a toxicity response.

Two high dose females were described as having bile duct hyperplasia, trace. This condition was not reported in the other groups. TB-I does not consider that magnitude of the apparent response to be sufficient to conclude that this common condition is an actual toxicity response.

B. Thyroid. In the rat chronic feeding study there were indications of possible compound related thyroid tumors and hyperplasia. In the main study, "parafollicular hyperplasia" was present in the controls (2) and the high dose group (2) without an indication of being a treatment related effect. In the females, however, there were three dogs affected with this condition and all were in the 500 ppm dose group. Because this lesion was not present in the high dose group, TB-I does not consider it to be compound related in females.

CONCLUSION. This study is classified as CORE GUIDELINE. No significant study deficiencies were recognized.