2-24-94

Micropike

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

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FEB 24 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id No.: 069001. Pyrethrum extract: Review of rat chronic feeding/carcinogenicity study (IRDC 1990) and mouse carcinogenicity study (IRDC 1990). Request for additional information concerning these studies.

TOX CHEM No.: 715
PC No.: 069001
Barcode No.: D190001, D190005
Submission No.: S438487

FROM: John Doherty 2/23/94
Section IV, Toxicology Branch I
Health Effects Division (7509C)

TO: Alan Dixon/Bruce Sidwell
Product Manager #53
Special Review and Reregistration Division (7505C)

THROUGH: Marion Copley, DVM, Section Head
Section IV, Toxicology Branch I
Health Effects Division (7509C)

I. CONCLUSION

The rat chronic feeding/carcinogenicity study (MRID No.: 415595-01) with pyrethrum extract was determined to be CORE MINIMUM. The chronic feeding aspects of the study indicated a NOEL and LEL of 100 and 1000 ppm based primarily on non-neoplastic liver pathology and hyperplasia in the thyroid of males. The study indicated compound associated increases in thyroid follicular cell adenomas in males at 1000 ppm and in males and females at 3000 ppm. The males were also associated with increases in skin keratoacanthomas at 3000 ppm. In addition, the presence in the high dose groups of adenomas in the liver in females, parathyroid adenomas in males and ovarian theca cell tumors will be referred to the Carcinogenicity Peer Review Committee for further evaluation. No additional series 83-5 chronic feeding/ carcinogenicity study data are required at this

time. Some additional information related to the findings in this study, however, is being requested by Toxicology Branch I. Refer to item III.A.3 below.

The mouse carcinogenicity study (MRID No.: 415594-01) with pyrethrum extract was determined to be SUPPLEMENTARY but is subject to upgrading. The registrant is being requested to provide certain additional information particularly related to the pathological findings in the lung as well as other information. Refer to item III.B.2.a, b. and c below.

II. Action Requested

The Pyrethrum Joint Venture has submitted a series 83-5 rat chronic feeding/carcinogenicity study and a series 83-2 mouse carcinogenicity study in order to satisfy the reregistration requirements for pyrethrum extract. These studies are identified in section IV below and were reviewed by the HFD contractor. The DERs are attached. The following comments apply.

III. Toxicology Branch Comments

A. Rat chronic feeding/carcinogenicity study.

1. The study is identified in section IV below.

2. The study was classified as CORE MINIMUM by the contractor. Toxicology Branch-I (TB-I) considers the study limitations indicated by the contractor (omission of analysis of alkaline phosphatase and cholesterol) to be minor. The testing laboratory should, however, be advised that future study protocols should include these parameters.

In addition to the conclusions presented in the DER, it is noted that the highest incidence of three additional tumors types are in the high dose group of males or females. These are: adenomas in the parathyroid of males, adenomas in the liver of females and ovarian theca cell tumors. TB-I does not at this time consider that the data available justifies a conclusion that these are compound related but will refer the incidence of these to the Carcinogenicity Peer Review Committee for further evaluation.

3. TB-1 requests the registrant to provide historical control data[1] on the spontaneous occurrence for the following tumor types:

-thyroid follicular adenomas and carcinomas (also of include incidence of non-neoplastic hyperplasia)
-keratoacanthoma
-liver adenomas and carcinomas
-ovarian theca cell tumors
-parathyroid adenomas and carcinomas

In addition, the registrant is requested to provide a literature survey and present to the Agency an overview on what evidence there is for chemical induction of keratoacanthoma in any species.

B. Mouse carcinogenicity study.

1. The study is identified in section IV below.

2. The study was classified as SUPPLEMENTARY but is potentially upgradable to an acceptable level. The following additional information pertaining to this study must be submitted.

a. The registrant must provide a comprehensive resummary of the pathological findings in the lung. This resummary should include a comprehensive table which lists separate columns for:

-the individual animal number for all animals on the study and the day of sacrifice for that animal;
-presence or absence of gross necropsy findings (notations such as N for nodules or M for masses can be used);
-the results of the first (single sectioning) reading (notations such as A for adenoma and C for carcinoma etc can be used), other types of tumors should also be indicated but clearly designated as to their origin;

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[1]These data must be submitted in tabular form indicating the date of the study, the supplier of the test animals and the incidence of tumors (both benign and malignant) and the number of animals examined for each organs listed above for each study. The studies should be all from the IPDC laboratory and be inclusive of the studies conducted 5 years prior to the study with pyrethrins and studies completed since completion of the pyrethrin study. Historical control data for relevant non-neoplastic lesions in the organs showing possible compound related neoplasms should also be provided.

-the results of the second (serial sectioning) reading (including the number of slides per animal actually read), this column should indicate by the notation A or C the presence of an adenoma or carcinoma;
-the number of distinct lung tumors per animal;
-the stage of development and the size estimate of the lung tumors.

b. Historical control data[2] for lung adenomas and carcinomas in the CD mouse strain tested.

c. More detailed information on the preliminary dose range finding study and the aborted attempt to conduct the definitive study at 7000 ppm.

C. Comparison of the neoplastic findings in the rat and mouse carcinogenicity studies.

Some general comments can be made based on an overview of these two studies.

-Thyroid and parathyroid adenomas or thyroid hyperplasia were not reported in the mouse study.
-One high dose group female in the mouse study was reported to have a carcinoma in the liver. This is considered rare in female CD-1 mice and may be of interest because the female high dose group rats had the highest incidence of liver tumors.
-Ovarian theca cell tumors were not reported in female mice.
-There was no indication of increased lung tumors in the male rats.

The above general comments may be of interest in preparing the Carcinogenicity Peer Review for pyrethrum extract.

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[2] Refer to footnote No. 1 on previous page for details for submitting historical control data.

IV. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
83-2. Carcinogenicity - mice International research and Development Corporation (IRDC), Study No.: 556-013, July 5, 1990.	Pyrethrum extract. provided by three producers - see DER for details.	415594-01	Pyrethrum extract was administered to Crl:CD-1 (ICR)BR mice at dietary levels of 0, 100, 2,500 or 5000 ppm corresponding to 13.8, 346 or 686 mg/kg/day for males and 16.6, 413 or 834 mg/kg/day for females. Equivocal findings for lung masses and nodules in the high dose male and female test group require additional information before their significance can be determined. NOEL and LEL (systemic) = 100 and 2500 ppm. At 2500 ppm: increased absolute and relative liver weight (20-25% for both sexes) and fatty change in liver.	SUPPLEMENTARY
83-5. Chronic feeding/carcinogenicity - rats International Research and Development Corporation, Study No.: 556-011, July 12, 1990.	Pyrethrum extract provided by three producers - see DER for details.	415595-01	Charles River CD strain rats. Dose levels tested: 0 (two control groups), 100, 1,000 or 3000 ppm corresponding to 4.37, 42.9 or 130 mg/kg/day in males and 5.39, 55.5 or 173 mg/kg/day in females. NOEL and LEL = 100 and 1000 ppm. At 1000 ppm: liver pathology (accentuated lobulation) in males only. At 3000 ppm: decreased body weight increased SGOT and SGPT (both sexes). Carcinogenic potential: increases in thyroid follicular cell adenomas and hyperplasia at 1000 ppm in males and 3000 ppm males and females. Increased acanthoma.	GUIDELINE

FINAL

010795

DATA EVALUATION REPORT

Pyrethrum Extract

Study Type: Combined Oral Oncogenicity/Chronic Toxicity Study in Rats

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

April 1993

Principal Reviewer: Laura Kolby Date 4/5/93
Laura Kolby, MPH

Independent Reviewer: William L. McLellan Date April 5 1993
Bill McLellan, Ph.D.

QA/QC Manager: Sharon C. Segal Date 4/5/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-35.1
Clement Number: 253
Project Officer: Caroline Gordon

Guideline Series 83 5 Combined Oral Oncogenicity
Chronic Toxicity Study in Rats

EPA Reviewer: Linnea J. Hansen, Ph.D.
Review Section IV, Toxicology Branch I,
Health Effects Division

Signature: Linnea J. Hansen
Date: 12/10/93

EPA Section Head: Marion Copley, D.V.M.
Review Section IV, Toxicology Branch I,
Health Effects Division

Signature:
Date: 2/23/94

DATA EVALUATION REPORT

DATA TYPE: Combined oral oncogenicity/chronic toxicity study in rats (Guideline Series 83-5)

TEST MATERIAL: Pyrethrum extract

SYNONYMS: None reported

PC NUMBER: 069001

MRID NUMBER: 415595-01

TOX CHEM. NUMBER: 715

STUDY NUMBER: Laboratory Project ID 556-011

SPONSOR: Pyrethrin Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC

TESTING FACILITIES: International Research and Development Corporation, Mattawan, MI

TITLE OF REPORT: Evaluation of Pyrethrum Extract in a Two Year Dietary Toxicity and Oncogenicity Study in Rats

AUTHOR: Edwin I. Goldenthal

REPORT ISSUED: /12/90

QUALITY ASSURANCE/COMPLIANCE: A signed Quality Assurance Statement dated July 12, 1990) and a list of Quality Assurance inspection dates were included. A statement of no confidentiality claim and a flagging statement were present signed, and dated. It was reported that the study was conducted under GLP compliance.

CONCLUSIONS: Doses administered in diet: 0 ppm (2 control groups), 100 ppm (males, 4.37 mg/kg/day; females, 5.39 mg/kg/day), 1,000 ppm (males, 42.9 mg/kg/day; females, 55.5 mg/kg/day), and 3,000 ppm (males, 130 mg/kg day; females, 173 mg/kg/day). Charles River CD rats.

Duration: 104 weeks

Systemic toxicity NOEL = 100 ppm for males (4.37 mg/kg/day); 1,000 ppm for females (55.5 mg/kg/day)

systemic toxicity LEL = 1,000 ppm for males (42.9 mg/kg/day) based on the increased incidence of accentuated lobulation of the liver in males; 3,000 ppm (173 mg/kg/day) for females based on decreased body weights and increased SGPT and SGOT values.

At 3,000 ppm male and female body weights were decreased, and SGPT and SGOT values were elevated in males. The increased incidence of accentuated lobulation of the liver in males was also attributed to compound administration. The increased incidence of spongiosis hepatis of trace severity in males may have been treatment-related.

At 1,000 ppm in males and at 3,000 ppm in males and females there was an increase in the incidence of thyroid follicular cell adenoma and hyperplasia. An increased incidence of skin keratoacanthoma in males was observed at 3,000 ppm as were increased liver tumors (females), parathyroid tumors — males and ovarian theca cell tumors in females.*

CORE CLASSIFICATION: This study is classified as Core Minimum for a combined chronic toxicity/oncogenicity study (83-5). Alkaline phosphatase and cholesterol were not analyzed.

A. MATERIALS, METHODS AND RESULTS

1. Test Article Description

Name: Pyrethrum extract

Active ingredients: Pyrethrin I and pyrethrin II

Molecular Formulas/Weights: Pyrethrin I $C_{21}H_{28}O_3$ 328.4 g
Pyrethrin II $C_{22}H_{28}O_5$ 372.4 g

Lot numbers: 3910 (McLaughlin Gormley King Co.),
344 (Pyrethrum Board of Kenya), and
"no lot number" (Office du Pyrethia en Rwanda

[Note: The test article was a mixture of equal parts of these three lots designated as FEK-99; prepared at Fairfield American Corp.]

Purity: 57.574% w/w (impurities were not identified)

Physical property: Amber liquid

Storage: Refrigerated in an airtight glass/stainless steel container

Stability: Samples of the bulk test article were analyzed by HPLC at weeks 1-4 and for every 4 weeks thereafter until week 104. No significant loss of the active ingredient, pyrethrins (pyrethrin I and pyrethrin II), was found throughout the test period. Test material concentrations ranged from 98.6%-105% of the week 1 sample (data extracted from Appendix A.).

* The significance (if any) of these relatively slight increases will be evaluated by the Carcinogenicity Peer Review Committee.

2. Diet Preparation

Diets were prepared weekly. The test compound was corrected for purity and an appropriate amount was added to a small amount of diet and mixed in a Hobart mixer to prepare a premix. To prepare the treated diets, premix was added to an appropriate amount of additional diet and mixed. Composite samples of each prepared diet (top, middle, and bottom of mixer) were collected, thoroughly mixed, and stored frozen. Samples were analyzed for concentration for weeks 1-4 and every 4 weeks thereafter. Homogeneity was tested prior to study initiation on 10 samples from each diet. Stability of the test compound in diets was also analyzed after 10 days of storage under simulated conditions of use.

Results- The pyrethrum extract was shown to be homogenous and stable in dietary mixtures for samples collected throughout the study. The mean percentages of target concentrations for 10 replicate samples from dietary samples at 100, 1,000 and 3,000 ppm were 96±5.5% (range, 85-107; only 1 sample was <93%), 99±2.2% (range, 96-104) and 98±2.2% (range, 95-101), respectively (data extracted from Appendix C)

The mean percentages of the day-0 concentration at 100, 1,000, and 3,000 ppm for samples after 10 days of storage at room temperature were 105%, 103%, and 106%, respectively (data extracted from Appendix D).

The mean concentrations of pyrethrum in diets (29 sampling intervals) at target levels of 100, 1,000, and 3,000 ppm were 101±5.0% (91-109%), 99±3.7% (91-106), and 98±4.0% (90-106 of nominal, respectively (data extracted from Appendix E).

3. Animals

Charles River CD rats (422 males and 420 females) were received from Charles River Laboratories (Portage, MI) and acclimated to laboratory conditions for 21 days prior to exposure to test diets. During acclimation, animals were observed at least twice daily for clinical signs and were given physical examinations weekly. Pretest screening on five rats/sex included microbiological screening, hematology, biochemistry, and gross necroscopies. Rats without normal weight gain between pretest days -8 and -1 were excluded from the study. After acclimatization, rats were randomized by body weight and assigned to the following study groups:

Group	Dietary Level (ppm)	No. of Animals Male	Female
1 (Control group 1)	0	60	60
2 (Control group 2)	0	60	60
3 (Low-LDT)	100	60	60
4 (Mid-MDT)	1,000	60	60
5 (High-HDT)	3,000	60	60

Animal room conditions included a temperature of 72±3°F, a relative humidity of 55±15%, and a 12-hour light/dark cycle. Food (Certified Rodent Chow #5002, Ralston Purina Co.) and water were provided ad libitum throughout the acclimation and study periods. Animals were caged three per cage during the first few days of acclimation and thereafter housed individually in wire-mesh cages.

4. Statistical Analyses

Body weight, food consumption, organ weight, hematologic, biochemical, and urologic data were analyzed using two-tailed statistical analyses with levels of significance set at $p < 0.01$ and $p < 0.05$. One-way analysis of variance, Bartlett's tests for homogeneity of variance, the appropriate T-statistic for equal or unequal variance (Steel and Torrie, Ostle), Dunnett's multiple comparison tables, and nonparametric analysis by rank (Conover and Iman) were used.

Nonparametric analysis (Conover and Iman method) was employed with total bilirubin, urine specific gravity, urine volume, creatinine phosphokinase, alanine aminotransferase, creatinine and aspartate aminotransferase data. Levels of significance were set at $p < 0.01$ and $p < 0.05$.

Tumor incidence data were analyzed using the method described by Huff, the life table test, the Hoel-Walburg incidental tumor test, Fisher's exact test, and the Cochran-Armitage trend test.

Data were analyzed by separate comparison with each control group. The study authors considered effects to be real only when significant compared to both controls. The reviewers considered effects to be treatment related if they were statistically significant when compared to both control groups and possibly treatment related if they were statistically significant when compared to one control group.

5. General Observations

(a) Mortality/moribundity/survival: All animals were examined twice daily for mortality or morbidity.

Results- Survival was not adversely affected by pyrethrum treatment and was adequate for the assessment of carcinogenicity; survival was ≥77% at 78 weeks and ≥28% at 104 weeks. At 78 weeks, survival in male groups ranged from 82% to 92% and in female groups from 77% to 88%. At termination, survival was 40%, 55%, or 60% for males receiving 100, 1,000, or 3,000 ppm, respectively, and 33%, 50%, or 53% for females at the same dose levels. Survival was 57% and 50% in control groups of males and 50% and 28% in control groups of females.

(b) Clinical observations: All animals were examined at least once daily for clinical signs of toxicity. In addition, each animal was given a detailed clinical examination including palpation for masses weekly.

Results- No treatment-related clinical effects were observed. Sporadic effects observed among all groups included subcutaneous masses, alopecia, decreased defecation, and anogenital staining.

(c) Body weights/body weight gains/food consumption/test material intake: Individual body weights were recorded weekly before treatment, weekly during treatment weeks 1-14 (months 1-3), and biweekly thereafter. Food consumption and test article intake were determined at the same intervals (starting at treatment week 1). Test article intake was calculated using food consumption and body weight data. Weight gain data were not derived.

Results- Body weight: Table 1 presents mean body weights and body weight gains at selected study intervals.

- 1,000 ppm: Females treated at 1,000 ppm had statistically significant decreases in mean body weight (compared to group control 2) which occurred at sporadic intervals from week 1 to week 54 and did not appear to be treatment-related.

- 3,000 ppm: Males treated at 3,000 ppm had statistically significant decreases in mean body weight, compared to both control groups, between weeks 2 and 76 (2% decrease at week 2, 7% decrease at week 76, vs control group 2). Females treated at 3,000 ppm had statistically significant decreases in mean body weights, compared to both control groups, consistently between weeks 5 and 76; thereafter differences were significant when compared to control group 2 (5% decrease at week 5, 12% decrease at week 76, vs. control group 2). These decreases in body weight at 3,000 ppm were considered to be treatment related in both sexes. The lack of statistical significance for male body weight decreases in the 3,000-ppm group at the 78-104 week interval may have been due to high mortality. At 3,000 ppm, mean body weight gain (vs. control 2) for males was 87% of control at 52 weeks and 96% of control at 104 weeks; mean body weight gain (vs. control 2) for females was 75% of control at week 52 and 73% of control at week 104.

Food consumption: Mean food consumption (g/animal/day) data at selected intervals are summarized in Table 2.

- 1,000 ppm: Statistically significant decreases in food consumption at 1,000 ppm, compared to one or both control groups, were sporadically seen in males at 6-102 weeks and

females at 1-102 weeks but did not appear to be treatment-related.

- 3,000 ppm: Statistically significant decreases in food consumption at 3,000 ppm, compared to one or both control groups, were frequently seen in males and females at weeks 1-102.

- Average daily food consumption data in g/kg/day from initiation to week 78 are provided in Table 2. At 1,000 ppm the average consumption was decreased by 1.2-2.7% in males and by 1-4% in females when compared to each control group. At 3,000 ppm it was decreased by 4.3-5.7% in males and by 3.1-6.0% in females. The decreases in food consumption at 3,000 ppm were considered to be possibly compound related.

Test material intake: Average daily test article intake (mg/kg/day) was calculated based on the weekly or biweekly mean food consumption data (g/animal/day), mean body weight data, and nominal dietary concentrations. The study author calculated that the intake of pyrethrum extract for males receiving diets containing 100, 1,000, and 3,000 ppm was 4.37, 42.9, and 130 mg/kg/day, respectively; the intake of pyrethrum extract for females receiving diets containing 100, 1,000, and 3,000 ppm was 5.39, 55.5, and 173 mg/kg/day, respectively.

(d) Ophthalmoscopic examination: All animals were examined during pretest and at study termination. A binocular indirect ophthalmoscope was used.

Results: No treatment-related effects were observed. Blepharitis, conjunctivitis, and keratitis were observed frequently at the termination among all test groups, including controls.

5. Clinical Pathology

Laboratory samples were collected for hematology, urinalysis, and clinical chemistry determinations from 15 randomly selected rats/sex/group at 6, 12, 18, and 24 months. Blood was drawn from the orbital sinus of fasted rats.

The parameters marked ("X") below were examined.

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Reticulocyte count (RETIC)
Red cell morphology
Nucleated erythroblasts
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
Coagulation: thromboplastin time (PT)
Activated partial thromboplastin time (APTT)

*Recommended by Subdivision F (November 1984) Guidelines

Results- There were no treatment-related changes in any of the hematology parameters reported.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium*
X Chloride*
Magnesium
X Phosphorus*
X Potassium*
X Sodium*

Enzymes

Alkaline phosphatase (ALP)
Cholinesterase (ChE)
X Creatine kinase
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

Other

X Albumin*
Albumin/globulin ratio
X Blood creatinine*
X Blood urea nitrogen
Cholesterol (total)*
X Globulin
X Glucose*
X Total bilirubin*
Direct bilirubin
X Total protein*
Triglycerides
Uric acid
Urea

*Recommended by Subdivision F (November 1984) Guidelines

Results- Table 3 presents mean SGPT and SGOT values at selected study intervals. SGPT values were significantly elevated in 3,000-ppm males at 6, 12, 18, and 24 months (significant relative to both controls at months 6, 12, and 18 and relative to one control group at 24 months). SGOT values were significantly elevated relative to both controls in 3,000-ppm males at 12, 18 and 24 months. The increases in SGPT and SGOT were seen in a subset of males in the high-dose group. Both parameters were increased 8-30 times above the normal values in the affected males. At 6, 12, 18, and 24 months, 3, 6, 9, and 6 of the 15 high-dose males tested had extreme levels of the enzymes. For some male animals (#23256, #28[illegible]6, #28273), the

increases correlated with liver weight at terminal sacrifice. There is a possible correlation with the gross finding of accentuated lobulation, but no microscopic correlations were evident when individual animal data were checked. Females were not affected. No other clinical chemistry parameters were adversely affected by dosing.

(c) Urinalysis

Urine was collected over a 24-hour period while fasted animals were housed in metabolism cages. The parameters marked ("X") below were examined.

X Appearance*	X Sediment (microscopic)	X Bilirubin*
X Volume*	X Protein*	X Blood*
X Specific gravity*	X Glucose*	X Nitrite
X pH*	X Ketones*	X Urobilinogen

*Recommended by Subdivision F (November 1984) Guidelines

Results- There were no treatment-related changes in urinary parameters.

7. Sacrifice and Pathology

All animals that died or were sacrificed moribund or sacrificed by design (week 104) were exsanguinated and necropsied. The tissues marked ("X") below were preserved and stained for histologic examination in all groups. The tissues marked ("X") were also examined histopathologically for the control and high-dose groups (only the sections from the kidneys, liver, lungs, thyroid gland, and tissues with macroscopic abnormalities were examined histopathologically for the other groups). The organs marked ("XX") were weighed for animals at scheduled sacrifice.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	XX Brain* (3 levels)
X Salivary glands*	X Heart*	X Peripheral nerve* (sciatic nerve
X Esophagus*	X Bone marrow*,#	X Spinal cord* (three levels
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	X Eyes* (optic nerve
X Jejunum*	X Thymus*	
X Ileum*		Glandular
X Cecum*	Urogenital	
X Colon*		XX Adrenals*
X Rectum*	XX Kidneys*	Lacrimal gland
XX Liver*	X Urinary bladder*	X Mammary gland
Gallbladder*	XX Testes*	X Thyroids*
X Pancreas*	X Epididymides	X Parathyroids*
	X Prostate*	Harderian glands
Respiratory	X Seminal vesicle	Zymbal's glands
	XX Ovaries*	
X Trachea*	X Uterus*	
X Lungs*	X Vagina and cervix	

Nasopharynx
Larynx

Other

X Bone (sternum and femur)*
X Skeletal muscle*
X Skin*
X All gross lesions and masses*

*Recommended by Subdivision F (November 1984) Guidelines
#Scheduled sacrifice animals only

(a) Organ weights

Table 4 summarizes absolute and relative liver weight data.

A statistically significant elevation, relative to control group 2, in mean liver-to-body-weight ratios was noted in males rats at 100 ppm (117% vs. control 2) and 3,000 ppm (117% vs. control 2) and in female rats at 3,000 ppm (117% vs. control 2). Since absolute liver weights were not significantly increased and liver-to-body-weight ratios were not affected at 1,000 ppm, the elevations in liver-to-body-weight ratios at 100 and 3,000 ppm were probably due to a slight decrease in mean body weights. No effects of dosing were observed for other organs.

(b) Macroscopic pathology

Table 5 summarizes macroscopic pathology data.

The incidence of accentuated lobulation of the liver was significantly elevated in male rats at 1,000 ppm (28% of total) and 3,000 ppm (23% of total) when compared to both control groups (10% of total - control 1, 13% of total - control 2) and was mostly mild to moderate in degree; severity of lobulation did not increase with dose. This increase was considered to be compound related. Other gross findings were those commonly found in rats of this strain for example, light colored foci in the lungs of both sexes, malocclusion /missing/broken teeth in both sexes, cloudy/opaque corneas in both sexes, and small testes and ulceration of the soft tissue of the feet in males. The incidences were low and did not increase with dose.

(c) Microscopic pathology

(1) Nonneoplastic lesions: The incidence of bile duct hyperplasia was significantly elevated in females at 1,000 and 3,000 ppm. relative to both control groups but did not appear to be treatment-related. The incidence of spongiosis hepatis of trace severity was increased in males (21/60) at 3,000 ppm ($p < 0.05$, chi square, relative to both control groups - 11/60 control 1, 10/60 control 2). The finding may represent very slight toxicity to the liver since the enzyme changes were also seen only at the high dose and only in males; however,

correlation between enzyme levels and histopathology in individual animals is not consistent.

Table 6 summarizes nonneoplastic lesions. There were no other unusual compound-related findings, either in survivors or decedents.

(2) Neoplasms: The incidences of selected preneoplastic and neoplastic lesions are summarized in Table 7. Several types of lesions, possibly related to compound administration, were noted.

Skin: The incidence of skin keratoacanthomas was significantly elevated in male rats at 3,000 ppm relative to both control groups (control 1 - 4/60, control 2 - 5/60, 3,000 ppm - 14/60); females were not affected. The percent incidence at low and mid doses could not be determined because skin was not collected from all the animals. Although the toxicological significance of this finding is unclear, the increase in skin keratoacanthomas may be attributed to compound administration. These tumors are benign, encapsulated, and self-limiting; they do not progress to malignancy like some other adenomas.

Thyroid: All thyroid lesions were subjected to peer review evaluation; some follicular adenomas were reclassified as hyperplasia and some follicular carcinomas were reclassified as follicular adenomas. The original pathology data were not presented in the study. The incidence of thyroid hyperplasia was significantly elevated ($p < 0.05$, control group 2) in males at 1,000 ppm (5/59) and significantly elevated ($p < 0.05$, both control groups) in males at 3,000 ppm (7/60). The incidence of thyroid follicular cell adenoma was also significantly elevated ($p < 0.05$, control group 2) in males at 1,000 ppm (5/59) and 3,000 ppm (5/60). The incidence of thyroid follicular cell adenoma was elevated ($p = 0.029$, both control groups) in females at 3,000 ppm (5/60). Historical data from 1983 onward; 10 studies) for thyroid tumors from IRDC Laboratories were provided; the average incidence of thyroid follicular cell adenoma was 2.2% (range, 0.00-6.78%) in males and 0.08% (range 0.00-1.67) in females. Data from other Charles River rat studies were compiled and provided by Lang; the incidence of follicular adenoma of the thyroid in controls ranged from 0% to 7.25% in males and from 0% to 4.29% in females. Although the increase in incidence of thyroid follicular cell adenomas and hyperplasia is minimal, it is probably real since it is seen in both sexes, is statistically significant, and is accompanied by increased hyperplasia and is outside the historical control range. In males, the increase over historical controls is minimal when compared to the incidence data from other labs (8.3% vs. 7.25%; 6.78% from this lab). However, looking at the historical control data for females from this lab, the highest percent incidence is 1.67% which is considerably lower than the incidence at high dose (8.3%) in this study. The data from other labs showed a similar pattern (max. % incidence for females of follicular cell adenoma was 4.29%). Furthermore,

the highest incidences represented unusual cases, whereas in the majority of studies the incidences were 0% - 3.5%. It is not uncommon that a higher incidence of thyroid tumors is found in males. It should also be noted that there was no increase in the incidence of follicular carcinoma.

There are some other pesticides that cause low incidences of thyroid tumors like this one and which cause the tumors by an indirect mechanism that may involve liver effects. The relation of liver effects to thyroid tumor formation is not so clear in this study since there was no clear hypertrophy and no liver effects in females, and since thyroid hormone levels were not determined.

Other sites: In addition, the incidences of parathyroid adenoma in males at 3,000 ppm and of ovarian theca cell tumors in females at 3,000 ppm were elevated. However, these increases were not statistically significant and were not attributed to compound administration. The usual types of tumors commonly observed in Sprague-Dawley rats were also present, including pheochromocytoma of the adrenal medulla and pituitary adenomas. The incidence of hepatocellular adenoma was significantly decreased for females at 3,000 ppm relative to control group 1, and the incidence of hepatocellular adenoma was significantly decreased for males at 3,000 ppm relative to control group 1. These changes in the incidence of hepatocellular adenoma were not considered to be biologically significant but will be discussed by the HED Cancer Peer Review Committee

B DISCUSSION

The study was adequately conducted and reported.

The reviewers agree with the study author's conclusion that survival, hematology, and urinalysis were not affected by dietary exposure to pyrethrum extract at doses up to 3,000 ppm. Clinical chemistry parameters with the exception of liver enzymes (see below) were also not affected.

Body weights were significantly decreased in males and females at 3,000 ppm; statistically significant decreases in body weight were not sustained throughout the entire study but were sustained throughout much of the study. There was some indication that the liver was a target organ as shown by the compound-related effects in males at 3,000 ppm: SGPT and SGOT values were elevated and there was an increased incidence of accentuated lobulation of the liver at 1,000 and 3,000 ppm. No treatment-related liver effects were observed in females - males were more sensitive for liver effects.

The increased incidence of spongiosis hepatis, which was significantly elevated in males at 3,000 ppm, is not clearly treatment-related but may represent a slight histopathological effect since male liver is clearly a target as determined by enzyme levels and gross pathology.

Dosing was adequate to assess the oncogenic potential as demonstrated by signs of toxicity at 3,000 ppm (body weight decreases at 3,000 ppm in males and females, and SGPT and SGOT value increases and lobulation of liver at 3,000 ppm in males). Under the conditions of this study, at 3,000 ppm there was an increased incidence of male skin keratoacanthomas, and small but statistically significant ($p < 0.05$) increased incidences of thyroid follicular cell hyperplasia and thyroid follicular cell adenomas were observed in both sexes. Also, at 1,000 ppm, the incidences of male thyroid gland follicular adenoma and hyperplasia exhibited statistically significant ($p < 0.5$) increases. Tumor incidence slightly exceeded the historical control range, although other lab control data show slightly higher incidences.

The NOEL for systemic effects is considered to be 100 ppm for males and 1,000 ppm for females.

The LEL for systemic effects is considered to be 1,000 ppm for males (42.9 mg/kg/day) based on the increased incidence of accentuated lobulation of the liver in males and 3,000 ppm (173 mg/kg/day) for females based on decreased body weights and increased SGPT and SGOT values.

Core Classification: Core-Minimum. Alkaline phosphatase and cholesterol blood levels were not determined.

TABLE 1. Mean Body Weight and Body Weight Gain at Representative Intervals for Rats Fed Diets Containing Pyrethrum Extract for 2 Years[a,b,c]

Dietary Level (ppm)	Allocation	26 wks	52 wks	78 wks	104 wks
		Mean Body Weight (g ± S.D.):			
			Males		
Control group 1	287±9.7	612±44.8	705±63.1	740±93.8	650±112.0
Control group 2	286±9.6	617±49.1	709±66.3	716±82.7	611±96.7
100	287±9.7	607±48.8	700±75.4	712±109.8	595±99.1
1,000	285±9.7	607±56.9	695±81.8	712±100.7	609±116.4
3,000	285±9.6	570±50.9*	653±67.9*	683± 92.3	596±103.4
			Females		
Control group 1	180±10.6	336±40.5	431±63.9	456±96.2	431±92.4
Control group 2	182±10.6	350±37.2	437±51.1	475±80.5	510±120.4
100	180±10.6	348±39.2	444±67.3	472±88.9	480±102.3
1,000	180±10.6	326±40.1†	410±68.6‡	459±87.3	440±116.7
3,000	183±10.4	311±32.7*	373±49.6*	415±67.8†	422±79.4‡
		Mean Body Weight Gain (g)			
			Males		
Control 1		325	418	453	363
Control 2		331	423	430	325
100		320 (98%, 97%)	413 (99%, 98%)	425 (94%, 99%)	308 (85%, 95%)
1,000		322 (99%, 97%)	410 (98%, 97%)	427 (94%, 99%)	324 (89%, 100%)
3,000		285 (88%, 86%)	368 (88%, 87%)	398 (88%, 93%)	311 (86%, 96%)
			Females		
Control 1		156	251	276	251
Control 2		168	255	293	328
100		168 (108%, 100)	264 (105%,104%)	292 (106%,100%)	300 (120%, 91%)
1,000		146 (94%, 87%)	230 (92%, 90%)	279 (101%, 95%)	260 (104%, 79%)
3,000		128 (82%, 76%)	190 (76%, 75%)	232 (84%, 79%)	239 (95%, 73%)

[a]Data were extracted from Table 3, pp. 91-98.
[b]Numbers in parentheses indicate percentage of control 1 and 2 values.
[c]Mean body weight gain was calculated by the reviewers.

*p ≤ 0.01 for both control groups
†p ≤ 0.01 for control group 2
‡p ≤ 0.05 for control group 1

TABLE 2. Mean Food Consumption (g/animal/day) and Average Food Consumption (mg/kg/day) at Representative Intervals for Rats Fed Diets Containing Pyrethrum Extract for 2 Years[a]

Dietary Level (ppm)	Mean Food Consumption (g/animal/day ± S.D.) at Interval: 1 wk	26 wks	52 wks	78 wks	104 wks	Average Food Consumption (mg/kg/day) through wk. 78
			Males			
Control 1	24.4±1.41	25.5±1.59	25.6±2.49	27.0±3.47	23.9±3.66	25.8
Control 2	24.6±1.64	25.1±2.77	25.6±2.32	25.5±3.93†	23.7±5.05	26.2
100	24.9±1.59	25.8±2.52	26.1±2.91	26.6±5.65	26.5±3.57	25.8
1,000	24.8±1.67	25.6±2.25	26.4±2.98	25.2±3.76†	23.1±5.09	25.5
3,000	23.4±1.42*	24.5±2.97†	26.1±2.16	25.6±3.65†	21.5±5.45	24.7
			Females			
Control 1	17.4±1.42	20.0±2.65	20.6±2.66	20.7±5.56	17.6±6.99	19.5
Control 2	17.8±1.58	20.9±2.19	21.5±2.61	21.7±3.61	20.7±5.58	20.1
100	18.1±3.56	20.5±2.53	22.3±2.84§	20.1±4 78	21.7±5.11	19.9
1,000	16.8±1.75†,¶	19.8±2.37‡	20.2±3.05	20.4±3.64	20.0±4.49	19.3
3,000	15.9±1.88*	19.1±2.36¶	20.5±2.88	19.9±3.91‡	20.0±4.71	18.9

[a]Data were extracted from Table 4, pp. 101-108, from Table 5, pp. 109-116 and from p. 31.

*$p < 0.01$ both control groups
†$p < 0.05$ control group 1
‡$p < 0.05$ control group 2
§$p < 0.01$ control group 1
¶$p < 0.01$ control group 2

TABLE 3. Blood Biochemistry Values (Mean ± S.D.) in Rats Fed Diets Containing Pyrethrum Extract for 2 Years[a,b]

Parameter/ Interval (months)	Dietary Level (ppm)				
	Control 1	Control 2	100	1,000	3,000
			Males		
Asparate Aminotransferase (IU/l)					
6	88±28.7	83±20.5	82±17.9	84±19.7	228±359.7
12	85±23.5	86±34.5	85±28.9	83±19.1	307±295.4*
18	127±94.6	117±47.3	133±35.4	134±84.3	826±842.2*
24	64±22.0	88±42.2	101±92.1	74±16.7	382±418.9*
Alanine Aminotransferase (IU/l)					
6	38±19.1	35±8.7	35±10.9	38±11.0	213±384.8†
12	37±10.4	43±23.5	46±26.3	40±12.2	381±459.2†
18	39±23.8	51±52.7	41±12.6	67±100.1	1294±1282.3*
24	32±11.1	51±43.1	51±70.7	36±8.6	551±678.4‡
			Females		
Asparate Aminotransferase (IU/l)					
6	81±26.2	82±35.1	84±30.9	89±43.4	96±69.3
12	80±18.3	74±13.8	87±22.2	80±23.6	81±39.7
18	90±15.3	94±26.6	93±16.8	89±17.2	138±152.0
24	244±686.8	68±27.8	66±22.2	74±30.8	96±97.6
Alanine Aminotransferase (IU/l)					
6	42±25.1	47±21.9	43±22.3	44±21.8	58±64.6
12	46±16.0	43±9.0	44±15.1	45±20.-	45±27.2
18	39±10.9	36±8.6	33±8.8	32±8.1	106±171.6
24	119±323.1	34±14.0	29±10.9	35±9.6	73±83.3

[a]Data were extracted from Table 7, pp. 129-144.
[b]N=15 (except for control group 1 males at 24 months N=14)

*p<0.01 for both control groups
†p<0.05 for both control groups
‡p<0.01 control group 1

TABLE 4. Absolute/Relative Liver Weight Data in Rats Fed Diets Containing Pyrethrum Extract for 2 Years[a,b]

Dose (ppm)	Males	Females
	Absolute Liver Weight (grams)	
Control 1	24.31	16.99
Control 2	22.01	19.69
100	24.72 (102%, 112%)	18.25 (107%, 93%)
1,000	24.35 (100%, 111%)	17.75 (104%, 90%)
3,000	24.86 (102%, 113%)	18.69 (110%, 95%)
	Liver-to-Body-Weight Ratio (%)	
Control 1	3.83	4.18
Control 2	3.64	3.99
100	4.25* (111%, 117%)	3.86 (92%, 97%)
1,000	4.08 (107%, 112%)	4.09 (98%, 103%)
3,000	4.25* (111%, 117%)	4.67* (112%, 117%

[a]Data were extracted from Table 10, pp. 173-180.
[b]Numbers in parentheses represent: % control 1, % control 2.

*$p < 0.05$ for control group 2

TABLE 5. Incidence of Macroscopic Liver Lesions in Rats Fed Diets Containing Pyrethrum Extract for 2 Years[a]

Liver Lesions	Dietary Level (ppm): Control Group 1	Control Group	100	1,000	3,000
			Males		
Accentuated lobulation					
Mild	5	5	9	12	12
Moderate	1	2	2	4	2
Severe	0	1	0	1	0
Total	6/60	8/60	11/60	17/60*	14/60*
% Total	10%	13%	18%	28%	23%
			Females		
Accentuated lobulation					
Mild	4	5	1	8	4
Moderate	1	1	1	1	2
Severe	0	0	1	0	0
Total	5/60	6/60	3/60	9/60	6/60

[a]Data were extracted from Table 9, pp. 149-172.

*Significant at $p < 0.05$ as compared to both control groups by Chi-square analysis (as calculated by the reviewers)

TABLE 6. Incidence of Nonneoplastic Lesions in Rats (N=60) Fed Diets Containing Pyrethrum Extract for 2 Years[a]

Liver Lesions	Dietary Level (ppm)				
	Control Group 1	Control Group 2	100	1,000	3,000
			Male		
Bile duct hyperplasia					
Trace	17	12	11	21	12
Mild	11	16	16	13	10
Moderate	0	4	1	0	2
Total	28/60	32/60	28/60	34/60	25/60
Spongiosis hepatis					
Trace	8	7	8	7	13
Mild	2	3	0	3	6
Moderate	1	0	0	1	2
Total	11/60	10/60	8/60	11/60	21/60*
			Female		
Bile duct hyperplasia					
Trace	8	3	6	12	14
Mild	4	4	4	12	7
Moderate	1	0	1	0	1
Total	13/60	7/60	11/60	24/60*	22/60*
Spongiosis hepatis					
Trace	0	1	0	2	0
Mild	0	0	0	0	1
Moderate	0	0	0	0	0
Total	0/60	1/60	0/60	2/60	1/60

[a]Data were extracted from Table 11, pp. 1c1-240.

*Statistically significant at $p < 0.05$ for both control groups by Chi-square analysis, as calculated by our reviewers.

TABLE 7. Incidence of Preneoplastic and Neoplastic Lesions in Rats Fed Diets Containing Pyrethrum Extract for 2 Years[a,b]

	Incidence by Sex and Dietary Level (ppm)									
	Males					Females				
	control group 1	control group 2	100	1,000	3,000	control group 1	control group 2	100	1000	3000
Thyroid gland	(60)	(60)	(60)	(59)	(60)	(60)	(60)	(60)	(60)	(60)
Follicular										
Adenoma	2	0	3	5*	5*	0	0	2	3	5†
Carcinoma	0	1	1	2	2	1	2	0	0	1
Adenoma/carcinoma	2	1	4	7*	7*	1	2	2	3	6
Hyperplasia	2	0	2	5‡	7§	0	2	1	1	5
Parafollicular										
Adenoma	6	6	5	3	4	3	2	5	4	6
Carcinoma	2	2	0	2	0	2	0	1	0	0
Adenoma/carcinoma	8	8	5	5	4	5	2	6	4	6
Parathyroid	(53)	(55)	(56)	(57)	(56)	(49)	(50)	(45)	(50)	(55)
Adenoma	1	0	0	0	4	0	0	1	0	1
Carcinoma	0	0	0	0	0	1	0	0	0	0
Liver	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
Hepatocellular										
adenoma	6	1	0	3	3‡	0	1	0	1	5¶
carcinoma	1	0	0	0	1	1	0	0	0	0
Skin	(60)	(60)	(24)	(14)	(60)	(60)	(59)	(8)	(8)	60)
Keratoacanthoma	4	5	7	6	14†	0	0	1	1	0
Ovary	-	-	-	-	-	(60)	(60)	(3)	(1)	(60)
Theca cell tumor						0	0	0	0	4
Mammary gland	-	-	-	-	-	(60)	(60)	(33)	(27)	(60)
Adenoma						9	9	8	5	2
Fibroadenoma	-	-	-	-	-	21	23	13	12	13
Adenocarcinoma	-	-	-	-	-	4	9	9	10	10
Pituitary	(60)	(58)	(23)	(16)	(60)	(59)	(60)	(41)	(43)	(60)
Adenoma	40	40	23	16	35	55	56	41	43	50

[a]Data were extracted from Table 12, pp. 241-288; Table 13, p. 289, and Table 14, pp. 290-309.
[b]The numbers in parentheses are the numbers of animals with a specific tissue examined histologically.

*Statistically significant at $p \leq 0.05$ for control group 2 by the Fisher Exact Test
†Statistically significant at $p \leq 0.05$ vs. both control groups by the Fisher Exact Test
‡Statistically significant at $p \leq 0.05$ for control group 2 by Chi-square analysis (as calculated by reviewers)
§Statistically significant at $p \leq 0.05$ for both control groups by Chi-square analysis (as calculated by reviewers)
¶Statistically significant at $p \leq 0.05$ for control group 1 by Chi-square analysis (as calculated by reviewers)

APPENDIX

Study report page 39

Pyrethrin

Page 27 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

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

____ The document is not responsive to the request.

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

010793

FINAL

DATA EVALUATION REPORT

Pyrethrum Extract

Study Type:
Oncogenicity Study in Mice

Study Title:
Evaluation of Pyrethrum Extract in an Eighteen Month Dietary Oncogenicity Study in Mice

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Sara Lundgaard Date 10/19/93
Sara Lundgaard, M.S.

Independent Reviewer William L McLellan Date 10/19/93
William McLellan, Ph.D.

QA/QC Manager Sharon A. Segal Date 10/19/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Clement Number: 254
Work Assignment Number: 2-35.1
Project Officer: Caroline C. Gordon

010798

Guideline Series 83-2: Oncogenicity Study in Mice

EPA Reviewer: Linnea J. Hansen, Ph.D.
Review Section IV, Toxicology Branch I,
Health Effects Division

Signature: Linnea J Hansen
Date: 11/1/93

EPA Section Head: Marion Copley, D.V.M.
Review Section IV, Toxicology Branch I
Health Effects Division

Signature: Marion Copley
Date: 11-1-93

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity study in mice

TEST MATERIAL: Pyrethrum extract

P.C. NO.: 069001

TOX CHEMICAL NO: 715

MRID Number: 415594-01

STUDY NUMBER: 556-013

SPONSOR: Pyrethrin Joint Venture/Chemical
Specialties Manufacturers Association
1913 Eye Street, N.W.
Washington, D.C. 20006

TESTING FACILITY: International Research and Development Corporation
Mattawan, Michigan 49071

TITLE OF REPORT: Evaluation of Pyrethrum Extract in an Eighteen Month Dietary Oncogenicity Study in Mice.

AUTHOR: E.I. Goldenthal, Ph.D.

REPORT ISSUED: July 5, 1990

CONCLUSIONS: Pyrethrum extract was administered to male and female Crl:CD-1(ICR)BR mice (60/sex/group) at dietary levels of 0, 100, 2,500, or 5,000 ppm for 18 months. Average test material intakes were 0, 13.8, 346, and 686 mg/kg/day in males and 0, 16 6, 413, and 834 mg/kg/day in females.

Under the conditions of this study, there were no statistically significant, treatment-related increases in tumor incidences. There was equivocal evidence for increases in the incidence of lung masses and nodules without increases in neoplastic lesions in high-dose males and females. However, these findings will require additional information before their significance can be determined.

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NOEL for systemic toxicity = 100 ppm (13.8 mg/kg/day in males; 16.6 mg/kg/day in females)

LOEL for systemic toxicity = 2,500 ppm (346 mg/kg/day in males; 413 mg/kg/day in females) based on increased absolute/relative weight and pathological changes in the liver.

CORE CLASSIFICATION: Core Supplementary (upgradable). This study may be upgraded for Guideline Series 83-2 (oncogenicity study in mice) pending submission of historical control data for lung adenomas/carcinomas, number of slides of lung tissue examined per animal, comparison of lung tumor size in different dose groups, and detailed results of the range-finding study.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Pyrethrum extract

Batch number: FEK-99 -- consisting of equal parts of lot #3910 (McLaughlin Gormley King Co.), lot #344 (Pyrethrum Board of Kenya), and "no lot number" (Office du Pyrethia an Rwanda)

Purity: 57.574% w/w (reported by the sponsor, impurities were not identified)

Physical property: Amber liquid

Storage: Refrigerated in an airtight container

Stability: Stable for duration of use (as reported by the sponsor)

2. Diet Preparation and Analyses for Purity and Stability

Test diets were prepared weekly at constant target concentrations of 0, 100, 2,500, and 5,000 ppm (adjusted for compound purity using a factor of 1.7369). A premix was prepared by mixing appropriate amounts of the test material with small amounts of the diet. The premix was then blended with the appropriate additional amount of diet for 10 minutes using a Hobart twin-shell blender. Purina Certified Rodent Chow #5002 was used as the control diet and as the basal diet in the preparation of test diets.

Samples of the test diet were analyzed to verify concentration, stability, and homogeneity by extraction with petroleum ether and quantification using high-performance liquid chromatography. Fifty gram samples were collected weekly from the top, middle, and bottom of each diet and stored frozen until analyzed. The samples collected from study weeks 1-4 and every 4 weeks thereafter were analyzed for concentration. The other samples were stored frozen for possible future analyses. Actual concentrations ranged from 87% to 110% of

nominal in all samples. On several occasions, the concentrations did not reach protocol standards and the samples were reanalyzed. The values obtained upon reanalysis were averaged with the original values to calculate the actual concentration. Target concentrations and actual measured concentrations were as follows:

Nominal Concentration (ppm)	Percent Nominal Achieved (±SD)[a]	Coefficient of Variation[b]
100	97% ± 5.5%	5.7%
2,500	99% ± 4.7%	4.7%
5,000	99% ± 4.7%	4.7%

[a]Data extracted from study 556-013, Appendix D, p. 248.
[b]Calculated by the reviewers

Prior to study initiation, ten samples were collected from each dose level to assess homogeneity of mixing. Diets were found to be acceptably homogenous. The 100-ppm diet showed a 2.8% coefficient of variation in composition. The 2,500-ppm diet showed a 3.5% coefficient of variation in composition. The 5,000-ppm diet showed a 3.4% coefficient of variation in composition. (Coefficients of variation were calculated by the reviewers.)

Samples for the analysis of stability were taken prior to study initiation. A single composite sample for each dietary level was collected from the top, middle, and bottom of the mixers and stored at room temperature for 10 days. After storage, 95-98% of the day-0 measured concentration remained. Thus, the test material was stable in the diet under these conditions.

3. Animals

A total of 420 male and 419 female CD-1 mice were received from Charles River Laboratories, Portage, MI. The mice were acclimated for approximately 20 days prior to the initiation of dosing. Animals were housed individually in wire-mesh cages and identified by toe clippings. All mice were examined two or three times a day for signs of disease and abnormality and given weekly detailed physical examinations during the 6-day acclimation period. Weight gain during the first 7 days was also monitored. A total of 10 mice/sex were randomly selected for health screening. All of these mice were subjected to gross necropsy, and 5 mice/sex in these groups were used for viral screening. Mice that were considered healthy and showed normal weight gain were randomly assigned to five groups (60 mice/sex/group) so that group mean weights were comparable. At the initiation of dosing, the mice were approximately 55 days of age and body weight ranges were 27-35 g for males and 21-29 g for females.

Animal room conditions included a temperature of 72 ± 3°F, a relative humidity of 55 ± 15%, and a 12-hour light/dark cycle. Food and tap water were provided *ad libitum* throughout the acclimation and study periods; both were monitored for contaminants. Caging and sanitary conditions were reportedly maintained in accordance with the SOPs of the testing facility. Cages and cage racks were relocated within the room every 2 weeks throughout the study.

Mice were randomly assigned to the following test and control groups:

Group	Dietary Level (ppm)	Number of Animals Males	Females
1 (control)	0	60	60
2 (control)	0	60	60
3 (low dose)	100	60	60
4 (mid dose)	2,500	60	60
5 (high dose)	5,000	60	60

Rationale for dose selection: The dose levels were reportedly based on the results of a 96-day range-finding study. A previous mouse oncogenicity study with doses of 100, 3,500, and 7,000 ppm was terminated because of excessive mortality in the high-dose group within the first 2 weeks of the study. References or further details on these studies were not provided in this report.

4. Statistical Analyses

Body weight, food consumption, and absolute and relative organ weight data were analyzed by the following methods: Bartlett's test and analysis of variance (ANOVA) for homogeneity of variance; the appropriate t-statistic (for equal or unequal variance) for comparison of the treatment group means against the control means; and Dunnett's Multiple Comparison for determination of significant differences. Two control groups were used in this study and were compared independently to the treated groups. Only data that were significantly different from both control groups were considered biologically significant. Tumor incidence data were analyzed using the life table test, the incidental tumor test, the Cochran-Armitage trend test, and Fisher's exact test.

QUALITY ASSURANCE: A signed quality assurance statement (7/5/90) was provided. A signed GLP certification statement and a signed flagging statement were present. The study was conducted in compliance with OECD guidelines and GLP regulations.

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5. General Observations

(a) Mortality/moribundity/survival

Animals were observed for mortality/moribundity three times a day during the week, and twice a day on weekends and holidays.

Results - No apparent treatment-related effects on mortality were observed. Percent survival at study termination ranged from 58% in the mid-dose males and control females to 83% in the low-dose females. Two 5,000-ppm animals (one male and one female) were found dead during the first week of the study. The study author attributed these deaths to treatment, but no rationale was given for this conclusion; histopathological examination did not reveal the cause of death.

(b) Clinical signs

Observations were made for adverse clinical effects three times a day during the week and twice a day on weekends and holidays. Detailed physical examinations, including palpation for masses, were conducted once a week throughout the study.

Results - No treatment-related clinical effects were observed. The study author stated that all high-dose animals showed increased activity during study week 1 when their cages were tapped. This increased activity disappeared after the first week. Since this finding was not listed in the individual clinical observations, it was not possible to confirm its occurrence.

(c) Body weights/food consumption/test material intake

Body weights--Body weights were measured twice weekly during acclimation, once weekly for the first 14 weeks of the study, and every 2 weeks thereafter.

Results - Representative summary body weight data are presented in Table 1. No apparent treatment-related effects were observed. Statistically significant differences were noted in both treated males and females throughout the study, but these occurrences were sporadic and were only significant when compared to one control group, not both. These changes were generally 2 grams or

less (-5% of controls). Body weight gain data were not provided for this study.

Food consumption--Food consumption was measured weekly for the first 14 weeks of the study and once every 2 weeks thereafter.

Results - Table 2 summarizes selected food consumption data. No treatment-related effects were observed. Average food consumption values (g/animal/day) in the treated groups were not significantly different from controls over the duration of the study. Statistically significant differences were noted in both treated males and females throughout the study, but these differences were sporadic and were only significant when compared to one control group, not both. These changes were generally 0.5 grams or less than controls.

Test article intake--Actual achieved dosages (mg/kg/day) were calculated by the study author from body weight and food consumption data. Mean daily doses for the duration of the study for the low-, mid-, and high-dose males were 13.8, 346, and 686 mg/kg/day, respectively; mean daily doses for the duration of the study for low-, mid-, and high-dose females were 16.5, 413, and 834 mg/kg/day, respectively.

6. Clinical Pathology

Leukocyte differential counts were evaluated in control and high-dose animals using blood taken from 10 randomly selected animals/sex/group during study months 12 and 18. No information was provided regarding method of blood collection.

Results - No statistically significant changes in hematology parameters were reported.

7. Sacrifice and Pathology

All animals that died during the study or were sacrificed either moribund or at study termination (by carbon dioxide asphyxiation) were given a complete postmortem examination by a pathologist. Gross examination included the organs of the abdominal, thoracic, and cranial cavities both *in situ* and after dissection. Samples of tissues and organs indicated by an "X" below were taken from all control and high-dose animals, preserved in phosphate-buffered neutral formalin (eyes were preserved in glutaraldehyde fixative), and examined histopathologically. In addition, all tissue masses with regional lymph nodes and all gross lesions found in all animals were sectioned and stained. Liver, kidney, and lung in the low- and mid-dose groups were also stained and examined histologically. Organs indicated by "XX" below were weighed prior to fixation for all animals.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	XX Brain*
X Salivary glands*	X Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord (three levels)*
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	X Eyes (Optic nerve)*
X Jejunum*	X Thymus*	
X Ileum*		Glandular
X Cecum*	Urogenital	
X Colon*		X Adrenals*
X Rectum	XX Kidneys*	Lacrimal gland
XX Liver*	X Urinary bladder*	X Mammary gland*
X Gallbladder*	XX Testes*	X Thyroids*
X Pancreas*	X Epididymides	X Parathyroids*
	X Prostate	Harderian glands
Respiratory	X Seminal vesicle	
	X Ovaries	
X Trachea*	X Uterus*	
X Lung*		

Other
X Bone (femur)*
X Skeletal muscle
X Skin*
X All gross lesions and masses*

*Recommended by Subdivision F (November 1984) Guidelines

(a) Organ weights and body weight ratios

Table 3 summarizes absolute and relative liver weight data. Significant increases ($p<0.01$) in absolute liver weight, liver-to-body-weight ratios, and liver-to-brain-weight ratios were observed in the mid- and high-dose males and females when compared to both control groups. The mean absolute weight of liver was 23-26% greater than controls in both sexes receiving 2,500 ppm and about 35% greater than controls in the groups receiving 5,000 ppm pyrethrum extract. No other treatment-related changes in organ weights were observed.

(b) Gross Pathology

Table 4 summarizes selected data on macroscopic lesion incidences. Statistical analyses were not performed on these data. The combined incidences of lung masses and nodules were increased in high-dose animals (19/60 in high-dose males compared to 7/60 and 10/60 in male controls; 17/60 in high-dose females compared to 9/60 and 7/60 in female controls). The incidences of liver discoloration were increased in high-dose males and mid- and high-dose females (20/60 in high-dose males compared to 1/60 and 0/60 in male controls; 7/60 in mid-dose females and 16/60 in

high-dose females compared to 0/60 in both female control groups). These lesions were considered treatment related.

Other common findings included kidney cysts, granular kidneys, cloudy corneas, liver masses, enlarged seminal vesicles, ovarian and uterine cysts, and alopecia. These lesions occurred with similar incidences in control and dosed animals and were not considered to be treatment related.

(c) Microscopic Pathology

Nonneoplastic lesions: Table 5 summarizes selected data on incidences of microscopic nonneoplastic lesions of the liver.

Statistical analyses were not performed on nonneoplastic lesions. The incidence of vacuolar fatty change of the liver was elevated in mid- and high-dose males (14/60 in high-dose, 8/60 in mid-dose, and 1/60 in both control groups). This accumulation of fat in the liver could account for the increase in liver weight in males. However, an increase in liver weight also occurred in the females, but fat accumulation did not. Thus in females the increase in liver weights may have been due, at least in part, to microsomal enzyme induction, not fatty change. No other treatment-related changes in nonneoplastic lesions were observed.

Neoplastic lesions: Table 6 summarizes data on microscopic incidences of microscopic neoplastic lesions of the lung.

Statistical analyses were performed on all microscopic neoplastic lesions except for on the single section of lungs. When a single section of lung from each animal was examined, an increase in incidence of alveolar/bronchiolar adenomas and carcinomas was observed in high-dose females. Because of the small size of the tumors and the historic variability in incidence of these tumors, additional lung sections from animals that did not have a previously diagnosed lung tumor were examined in the high-dose and control females. Lung tissue was sectioned into 5μ slices and every 10th slide was stained and examined (number of slides examined per animal was not indicated). Upon reevaluation of the slides, additional female control animals were found to have neoplasms (11 animals, 13 animals); for high-dose females, three additional animals with lung neoplasms were identified. The reevaluation suggested that there was no apparent increase in incidence of lung neoplasms in treated animals (see Reviewer's Discussion, Section B). Historical control data of lung neoplasms was not provided for comparison.

The incidence of liver neoplasms in males of all groups was higher than in females, but the increased incidence did not correlate with treatment. Neoplasms at other sites occurred sporadically and generally at an incidence of less than 4%/group. Some of the more frequently occurring sporadic neoplastic lesions include, hematopoietic neoplasms (i.e., malignant

lymphoma/lymphocytic and granulocytic leukemia) and hepatocellular neoplasms (i.e., adenomas and carcinomas).

B. DISCUSSION

A review of the final report and supporting data indicate that the conduct of the study was adequate and the reporting of the results was accurate. The design of the study was judged adequate to fulfill the intent of the Subdivision F Guidelines.

Although it appeared that under the conditions of this study, there were no statistically significant, treatment related increases in tumor incidence in mice, additional information is required before this conclusion is made. The incidence of grossly observed lung masses was increased in both sexes at the high dose. Initial examination using one lung slice per animal showed that the incidence of alveolar/bronchiolar adenomas and carcinomas was increased in high-dose females. However, when additional slices were examined, this increase in incidence was no longer observed. The method of resolving this issue by re-examining additional slides of lungs of control and high-dose females may have introduced bias. The same number of slides should have been examined for lungs of all groups of animals (including low- and mid-dose groups) at least in females. In addition, the number of slides examined per mouse was not indicated, and data on the initial evaluation were not substantiated by individual animal records. Lung adenomas and carcinomas could not be evaluated separately for the data before additional lung slides were examined. No other treatment-related tumors were observed.

The IRDC laboratory historical control incidence was not provided for lung neoplasms in Crl:CD-1 mice. The spontaneous rate of neoplastic lesions in Crl:CD-1 mice has been published by Charles River Laboratories. At 18 months, the incidences of bronchiolar/alveolar adenoma and alveolar type II adenomas in 496 mice (8 groups) were 0-8.8% and 0-20.0%, respectively[1].

The reviewers request that the Registrant submit (1) historical control data for lung adenoma/carcinomas in mice from the testing lab (by individual study, ±2 or 3 years of this study, separate and combined adenoma/carcinoma incidences); (2) number of slides of lung tissue examined per animal in control and high dose groups; and (3) if possible, a comparison of lung tumor size in the different dose groups as the reported observations may have represented a difference in size of tumors but not incidence. In addition, it is requested that the Registrant submit (4) a summary of the range-finding study results and of the terminated mouse study, including separate male and female mortality data.

Dosing was adequate in the study. Guidelines state that the highest dose tested (HDT) must produce adverse effects in animals without substantially altering the lifespan of the animals. Statistically significant increases in absolute liver weight, liver-to-body-weight ratios, and liver-to-brain-weight ratios were observed in mid- and high-dose males and females. Increases in incidences of liver discoloration were observed in high-dose males and mid- and high-dose females. The incidence of vacuolar fatty

change of the liver was elevated in mid- and high-dose males. In high-dose females, only slight liver enlargement and discoloration were observed, which may have represented an adaptive liver response rather than overt toxicity. However, since the high dose (834 mg/kg/day) approached the limit dose of 1,000 mg/kg/day this was considered adequate.

Survival was acceptable in the study (73% and 70% in high-dose males and females, respectively). Sporadic, yet statistically significant increases in mean body weight and food consumption were observed, but these were not considered to be treatment related. No statistically significant changes in hematology parameters were reported.

The LOEL for chronic systemic toxicity was 2,500 ppm based on organ weight changes and microscopic pathology changes in the liver. The NOEL for chronic systemic toxicity was 100 ppm.

Classification: Core Supplementary (upgradable pending submission of requested information).

[1]Lang, P.L. Spontaneous Neoplastic Lesions in the Crl:CD-1®(ICR)BR Mouse. Charles River, 1991.

TABLE 1. Mean Body Weight at Representative Intervals in Mice Fed Pyrethrum Extract in the Diet for 18 Months[a]

Dietary Level (ppm)	Mean Body Weight (g ± S.D.) at Week: -1	5	20	30	46	56	68	78
					Males			
0	30±1.9	34±2.2	38±2.8	40±3.7	41±3.5	41±3.3	41±3.7	41±3.7
0	30±1.9	33±2.3	38±2.9	39±3.2	40±4.0	40±3.7	41±4.0	40±3.3
100	30±1.9	33±2.0	37±2.6	39±3.1	41±3.3	40±3.4	41±3.8	40±3.2
2500	30±1.6	34±1.7	37±2.3	39±2.5	40±3.0	40±3.7	40±3.2	40±3.2
5000	30±1.8	34±2.1	37±2.5	38±2.7*,**	40±3.0	39±3.1	39±3.6*,**	39±3.1
					Females			
0	24±1.5	27±1.6	30±2.3	32±2.7	33±2.9	34±3.3	34±3.6	34±3.3
0	24±1.6	27±2.0	30±2.6	32±3.6	34±4.7	35±4.4	35±5.3	35±5.3
100	24±1.5	28±1.7*,**	31±2.2*,**	33±2.6†	35±3.0†	35±3.1	35±3.3	36±3.7
2500	24±1.8	28±2.1*,**	30±2.3	32±2.5	34±2.9	34±3.0	34±3.5	35±3.7
5000	24±1.6	28±1.9†,‡	30±2.1	32±2.3	34±2.6	34±3.0	34±3.1	34±2.7

[a]Data extracted from Study No. 556-013, Table 3 (pp. 89-96), and Appendix G.
*Significantly different from Control Group 1, $p<0.05$.
†Significantly different from Control Group 1, $p<0.01$.
**Significantly different from Control Group 2, $p<0.05$.
‡Significantly different from Control Group 2, $p<0.01$.

TABLE 2. Mean Food Consumption at Representative Intervals in Mice Fed Pyrethrum Extract in the Diet for 18 Months[a]

Dietary Level (ppm)	Mean Food Consumption (g/animal/day ± S.D.) at Week: 1	5	20	30	46	56	68	78
				Males				
0	5.4±0.52	5.5±0.32	5.2±0.51	5.0±0.36	5.0±0.41	4.8±0.75	4.9±0.48	5.4±0.46
0	5.4±0.62	5.6±0.51*	5.2±0.57	5.2±0.45†	5.1±0.56	4.7±0.44	4.9±0.53	5.4±0.41
100	5.4±0.47	5.5±0.51	5.3±0.74	5.1±0.43	4.9±0.36**	4.7±0.37	4.9±0.49	5.6±0.39**
2500	5.3±0.38	5.4±0.41‡	5.4±0.59	5.2±0.41	5.2±0.51*	4.8±0.65	4.9±0.56	5.6±0.59**
5000	5.3±0.30	5.3±0.39†,‡	5.1±0.41	5.0±0.39‡	5.0±0.40	4.8±0.54	4.8±0.56	5.3±0.41
				Females				
0	5.0±0.57	5.5±0.80	5.4±0.83	5.2±0.88	5.1±0.91	5.1±0.97	4.9±0.72	5.5±0.96
0	5.3±0.75†	5.6±0.70	5.5±1.09	5.4±1.21	5.3±0.93	5.0±0.56	5.1±0.68	5.8±0.67
100	5.1±0.49**	5.3±0.73	5.5±1.07	5.2±0.75	5.0±0.76**	5.0±0.61	5.0±0.69	5.5±0.58**
2500	4.8±0.39*,‡	5.1±0.67†,‡	5.2±0.61**	5.2±0.64	5.0±0.49**	4.9±0.63	5.0±0.66	5.4±0.65‡
5000	5.0±0.62‡	5.4±0.62	5.2±0.60**	5.1±0.49**	4.9±0.57‡	4.8±0.84	5.1±0.77	5.5±0.66**

[a]Data extracted from Study No. 556-013, Table 4 (pp. 95-106), and Appendix H.
*Significantly different from Control Group 1, $p<0.05$.
†Significantly different from Control Group 1, $p<0.01$.
**Significantly different from Control Group 2, $p<0.05$.
‡Significantly different from Control Group 2, $p<0.01$.

TABLE 3. Mean Absolute and Relative Liver Weights Ratios (±S.D.) in Mice Fed Pyrethrum Extract in the Diet for 18 Months[a]

Organ	Dietary Level (ppm) 0	0	100	2,500	5,000
			Males		
Liver (g)	2.29 ± 0.352	2.33 ± 0.333	2.38 ± 0.398	2.89 ± 0.347†,‡ (125%)[b]	3.10 ±0.412†,‡ (134%)
Liver-to-body (%)	5.77 ± 0.775	5.81 ± 0.717	5.92 ± 1.010	7.30 ± 0.712†,‡ (126%)	7.91 ± 0.751†,‡ (137%)
Liver-to-brain (%)	4.33 ± 0.683	4.38 ± 0.678	4.50 ± 0.753	5.37 ± 0.654†,‡ (123%)	5.79 ± 0.673†,‡ (133%)
			Females		
Liver (g)	2.17 ± 1.016	2.18 ± 0.340	1.99 ± 0.409** (91%)	2.68 ± 0.792*,‡ (123%)	2.90 ± 0.464†,‡ (133%)
Liver-to-body (%)	6.20 ± 2.197	6.29 ± 0.710	5.68 ± 1.042‡ (91%)	7.48 ± 1.662†,‡ (120%)	8.46 ± 0.987†,‡ (135%)
Liver-to-brain (%)	4.01 ± 1.862	4.00 ± 0.678	3.65 ± 0.761** (91%)	4.88 ± 1.482*,‡ (122%)	5.40 ± 0.049†,‡ (135%)

[a]Data extracted from Study No. 556-013, Table 8 (pp. 136-139), and Appendix K.
[b]Numbers in parentheses represent percent of average control values.
*Significantly different from Control Group 1, p≤0.05.
†Significantly different from Control Group 1, p≤0.01.
**Significantly different from Control Group 2, p≤0.05.
‡Significantly different from Control Group 2, p≤0.01

TABLE 4. Incidence of Selected Macroscopic Findings (%) in Mice Fed Pyrethrum Extract for 18 Months[a,b,c]

Organ/ Lesion	Dietary Level (ppm) 0	0	100	2,500	5,000
			Males		
Lung Nodules/Masses	7 (11.7)	10 (16.7)	9 (15.0)	9 (15.0)	19 (31.7)
Liver Discoloration	1 (1.7)	0	2 (3.3)	1 (1.7)	20 (33.3)
			Females		
Lung Nodules/Masses	9 (15.0)	7 (11.7)	14 (23.3)	7 (11.7)	17 (28.3)
Liver Discoloration	1 (1.7)	0	0	7 (11.7)	16 (26.7)

[a]Data extracted from Study No. 556-013, Table 7 (pp. 117-133) and Appendix L.

[b]N=60 for each dose group

[c]The numbers in parentheses indicate percentage of animals in dose group with lesion.

Note: Statistical significance not determined.

TABLE 5. Incidence of Selected Nonneoplastic Lesions (%) in Mice Fed Pyrethrum Extract in the Diet for 18 Months[a,b]

Organ/ Lesion	Dietary Level (ppm)				
	0	0	100	2,500	5,000
			Males		
Liver Vacuolar change/fatty	1 (1.7)[c]	1 (1.7)	2 (3.3)	8 (13.3)	14 (23.3)
			Females		
Liver Vacuolar change/fatty	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a]Data extracted from Study No. 556-013, Table 9 (pp. 140-192) and Appendix M.

[b]N=60 for each dose group

[c]The numbers in parentheses indicate percentage of animals in dose group with lesion.

Note: Statistical significance not determined.

TABLE 6. Incidence of Selected Neoplastic Lesions in Mice Fed Pyrethrum Extract in the Diet for 18 Months[a,b]

	Dietary Level (ppm)									
	Males					Females				
Site/Lesion	0	0	100	2500	5000	0	0	100	2500	5000
Lung Alveolar/Bronchiolar Adenomas and Carcinomas										
Initial Evaluation:										
No. with Tumors	14	16	16	16	20	9	7	11	7	21
Percent Incidence	(23.3)	(26.7)	(26.7)	(26.7)	(33.3)	(15.0)	(11.7)	(18.3)	(11.7)	(35.0)
Additional Evaluation[c]:										
No. with Tumors	-	-	-	-	-	20	20	-	-	24
Percent Incidence						(33.3)	(33.3)			(40.0)
Lung Alveolar/Bronchiolar Adenomas										
Initial Evaluation:										
No. with Tumors	14	16	15	13	17	8	7	11	5	NR
Percent Incidence	(23.3)	(26.7)	(25.0)	(21.7)	(28.3)	(13.3)	(11.7)	(18.3)	(8.3)	
Additional Evaluation:										
No. with Tumors	-	-	-	-	-	19	-	-	-	22
Percent Incidence						(31.7)				(36.7)

TABLE 6 (Continued). Incidence of Selected Neoplastic Lesions in Mice Fed Pyrethrum Extract in the Diet for 18 Months[a,b]

	Dietary Level (ppm)									
	Males					Females				
Site/Lesion	0	0	100	2500	5000	0	0	100	2500	5000
Lung Alveolar/Bronchiolar Carcinomas										
Initial Evaluation:										
No. with Tumors	0	0	1	3	3	1	3	0	2	NR
Percent Incidence	(0)	(0)	(1.7)	(5.0)	(5.0)	(1.7)	(5.0)		(3.3)	
Additional Evaluation:										
No. with Tumors	-	-	-	-	-	3	-	-	-	2
Percent Incidence						(5.0)				(3.3)
Liver-Hepatocellular Carcinoma										
No. with Tumors	4	3	1	0	3	0	0		0	1
Percent Incidence	(6.7)	(5.0)	(1.7)	(0)	(5.0)	(0)	(0)		(0)	(1.7)
Liver-Hepatocellular Adenoma										
No. with Tumors	5	12	4	11	10	1	0	0	0	1
Percent Incidence	(8.3)	(20.0)	(6.7)	(18.3)	(16.7)	(1.7)	(0)	(0)	(0)	(1.7)

[a]Data extracted from Study No. 556-013, Table 10 and 11 (pp. 193-238) and Appendix L.
[b]N=60 for each dose group
[c]Additional sections of lung tissue were analyzed in control and high-dose females. The lung tumors from high-dose females were compared independently to the two control groups using the combined results from the initial data and additional data.
NR - Not Reported