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

002602

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: W. H. Miller/M. A. Mautz
Product Managers, Team No. 16
Registration Division (TS-767)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

Budd 3/18/83

THRU: Orville E. Paynter, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: "ORTHENE Technical (RE-12420): Lifetime Oral Carcinogenicity Study in Mice. No. 415-006; 2/24/82. EPA Accession Nos. 247717-247719; EPA Record No. 71364.

TOX Chem No. 2A

This study was conducted by International Research and Development Corporation, Mattawan, Michigan, for Chevron Chemical Company, Richmond, California. The study was reviewed for the EPA/HED/TB (TS-769) by a contractor, the MITRE Corporation, McLean, Virginia. A copy of MITRE's evaluation, approved by Toxicology Branch, and a summary of the most important observations, prepared by Toxicology Branch, are attached.

The most important finding was that female CD1 mice (strain used in this study), fed 1000 ppm of Technical Orthene for 2 years, had a higher incidence of hepatocellular carcinomas and hyperplastic nodules than did the controls. All of these carcinomas and most of the nodules were observed only at the terminal sacrifice.

Classification of this study: Core Minimum.

Krystyna K. Locke

Krystyna K. Locke, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

Attachment

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OPP:HED:TOX:K.LOCKE:sb 3/8/83 X71511 Rm 824 #m24

Summary of the Most Important Observations in the Study Entitled "ORTHENE Technical (RE-12420): Lifetime Oral Carcinogenicity Study in Mice" No. 415-006; 2/24/82. EPA Accession No. 247717-247719; EPA Record No. 71364.

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1. Female CD1 mice, fed 1000 ppm of Technical Orthene (acephate; highest level tested) for 2 years, had a higher incidence of hepatocellular carcinomas and hyperplastic nodules than did the controls. (Other levels of Technical Orthene fed were 50 and 250 ppm.)

- The incidence of hepatocellular carcinomas in the high-dose females and the controls was 15.8% and 1.3%, respectively. All of these hepatocellular carcinomas were observed at the terminal sacrifice. Statistical analyses of the significance of tumor incidence were not provided.

- The incidence of liver hyperplastic nodules (non-neoplastic lesions) in the high-dose females and the controls was 19.7% and 2.7%, respectively. Most of these nodules (14.5%) were observed at the terminal sacrifice.

- There were no hepatocellular carcinomas and no hyperplastic nodules in the mid-dose females.

- The incidence of hepatocellular carcinomas in the low-, mid- and high-dose male groups, and in the low-dose females, was either the same or lower than that observed in the controls.

- The incidence of hepatocellular adenomas was low in this study. For the low-, mid- and high-dose females, the incidence was 2.7, 0 and 3.9%, respectively. For each one of the male test groups, the incidence of hepatocellular adenomas was 1.3%. There were no hepatocellular adenomas in the male and female control mice.

- The incidence of other hepatic neoplastic lesions (hemangiosarcomas and hemangiomas) in the females, treated with 0, 50, 250 or 1000 ppm of Technical Orthene, was 1.3, 1.3, 0 and 2.6%, respectively. The corresponding values for the male groups were 0, 2.7, 0 and 1.3%, respectively.

2. The incidence of hepatocellular carcinomas in the historical controls (23 studies representing 1630 CD1 female mice) ranged from 0 to 6%.

3. There were dose-related non-neoplastic liver injuries (hypertrophy of hepatocytes, karyomegaly and intranuclear inclusion bodies, in the males and the females, in the groups treated with 250 or 1000 ppm of Technical Orthene. The highest incidence of these injuries occurred at the terminal sacrifice.

4. There were dose-related injuries in the lungs (dark pigmented alveolar macrophages, eosinophilic foreign bodies and alveolar hyalinosis) and in the nasal cavities (acute rhinitis), in all groups of mice receiving the test material. At the 1000 ppm level, the highest incidence of lesions was observed at the terminal sacrifice, in the males and the females.

5. Female mice in the 1000 ppm group had larger livers (relative weight), smaller kidneys and brains (absolute weight), and smaller ovaries (absolute and relative weights) when compared with the controls. Male mice in the 1000 ppm group has smaller livers and kidneys (absolute weight) when compared with the controls. The mean increase in liver weight of the females was statistically significant at the 5% level. The mean decreases in the weights of other organs, in both sexes, were statistically significant at the 1% level.

6. Male and female mice in the 1000 ppm group weighed 8-30% less during this study than did the controls and the mice exposed to 50 or 250 ppm of Technical Orthene. Most of the decreased weight gains were statistically significant at the 1% level.

7. Orthene Technical, at all levels tested, had no effect on the appearance of animals, behavior, food consumption, hematology, mortality and tissue pathology (other than liver an lung).

8. Classification of this study : Core Minimum.

Krystyna K. Locke

Krystyna K. Locke, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

OPP:HED:TOX:K. LOCKE:sb 3/8/83 X71511 Rm 824 #m24

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Tox. Chem. No. 2A

MRID not assigned
EPA Accession Nos. 247717-19
EPA Record No. 71364

1. Chemical or Chemicals

Acephate

(Orthene Technical, RE-12420)

2. Type or Formulation:

Technical (92.7%)

3. Citation or Citations:

Spicer, E.J.F. (Study Director), 1982. "Lifetime Oral Carcinogenicity Study in Mice." Testing Laboratory, International Research and Development Corporation (IRDC), Mattawan, Michigan. Study No. 415-006; 2/24/82. Submitted by Chevron Chemical Company.

4. Reviewed By:

Finis L. Cavender
Scientist
The MITRE Corporation
1820 Dolley Madison Boulevard
McLean, Virginia 22102
(703) 827-2978

Signature: Finis Cavender
Date: 9/17/82 (1ST DRAFT)
3/11/83 (FINAL COPY) 7/6/83

5. Approved By:

Signature: Phyllis J. Locke 3/14/83
Date: EPA/HED/TB (TS-769)

6. Discipline/Topic or Test Type:

This study has information pertinent to discipline toxicology, TOPIC CHRONIC FEEDING AND ONCOGENICITY.

This study relates to the Propcsed Guidelines data requirement 158.135, Reference Nos. 83-1 and 83-2. Federal Register/ Vol. 47, No. 227/November 24, 1982.

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7. Conclusions:

Charles River weanling CD-1 mice (75 per sex per group) approximately 4-weeks of age were fed Orthene Technical (RE-12420) for 2 years at levels of 0, 50, 250, and 1000 ppm in the diet. Chemical analyses revealed wide variations in the weekly preparation of diet so that it is not possible to know the exact dosage levels in this study. No differences were seen in mortality due to treatment for either sex. A significant reduction ($p \leq 0.01$) in body weight as compared to control values was observed for both males and females in the 250 and 1000 ppm groups. A significant reduction ($p \leq 0.01$) in food consumption (mg/mouse/day) as compared to control value was noted for the 1000 ppm mice and for weeks 95-104 for the 250 ppm mice. These may be related to palatability of the diet since there were no significant differences on a mg/kg/day basis. An unexplained decrease in food consumption was noted for female mice for week 4 of the study and for all groups for week 43. No remarkable changes were noted in the hematological data reported (measured at study termination) although a considerable number of data points were lost due to an equipment malfunction. Approximately one-third of the animals were necropsied at the terminal sacrifice prior to receiving an authorization from the sponsor to evaluate organ weight as a toxicological end-point. A significant reduction ($p \leq 0.01$) in the

absolute brain weight as compared to control values was noted for the 1000 ppm female mice. This and body weight considerations indicate that the maximum tolerated dose (MTD) may have been exceeded at the 1000 ppm level. An increase in the relative brain weight of both male and female mice in the 1000 ppm group is reflective of decreased body weight in these animals.

Of the proliferative changes noted in the study, the only treatment-related changes were an increase in liver hyperplastic nodules and an increase in hepatocellular carcinomas in the 1000 ppm female mice. The study provided no statistical analyses of the significance of tumor incidence.

Of the non-neoplastic lesions, changes in the liver and lung were of particular interest. A clear dose-response in liver injury (i.e., hepatocyte hypertrophy, karyomegaly, intranuclear inclusion bodies, and accumulation of mononuclear inflammatory cells) was noted in the 250 ppm and 1000 ppm groups. The 50 ppm group was free of treatment-related liver injury.

Respiratory tract lesions found in all groups of mice consisted of the accumulation of pigmented alveolar macrophages, eosinophilic crystalloid (foreign) bodies, alveolar hyalinosis, and acute rhinitis. The incidence of pigmented macrophages increased with time and with increasing dosage. The incidence of eosinophilic foreign

bodies (at the terminal sacrifice) was 28% in males and 32% in females in the 1000 ppm group, while the control, 50, and 250 ppm male and female groups each had the incidence of 9-10, 8-12, and 11-12, respectively. The presence of these eosinophilic foreign bodies had not been observed previously in control animals by the pathologists involved in the study. Their presence raises questions as to the health of the animals and/or the conduct of the study.

In summary, the reviewer concluded:

For Oncogenicity:

1) The MTD for Orthene Technical may have been exceeded for Charles River CD-1 female mice at 1000 ppm because of the absolute brain weight decrease observed in these animals and because the high dose animals exhibited a 30% decrement in body weight.

2) Using a 10% decrement in body weight as the criterion for the MTD, the 250 ppm group did not reveal any tumorigenicity as compared to controls.

3) An increased incidence of hepatocellular carcinoma was evident in female mice given 1000 ppm Orthene Technical only at the terminal sacrifice; therefore, Orthene Technical does not increase the onset of cancer.

For Chronic Toxicity:

1) The clinical health NOEL for Orthene Technical is 50 ppm and the LEL is 250 ppm based on the body weight data.

2) The microscopic pachology NOEL of Orthene Technical is 50 ppm and the LEL is 250 ppm based on the liver changes noted in both male and female mice.

CORE CLASSIFICATION:

For Oncogenicity: Core Minimum.

For Chronic Toxicity: Core Minimum.

Note: In this study the diet analyses revealed poor quality control in the diet preparation and the possibility of the diets being switched between the groups on study. In this case, however, the mean values of dietary concentrations, the increase in severity in the liver and lung lesions with time and with increasing dose, and the pattern of body weight change, indicate that potential problems in quality control were not serious enough to alter the development of these changes.

8. Materials and Methods:

This study was conducted at the International Research and Development Corporation.

Experimental Design

Groups of 407 male and 407 female weanling CD®-1 mice (approximately 4-weeks old) purchased from The Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts (the specific facility was not designated) were received on June 28, 1978 and conditioned for 1 week. During the conditioning period, food and body weight measurements were recorded. Three hundred male (22-26g) and 300 female (18-22g) mice with no physical abnormalities were selected

randomly and initiated into the control and three dosage-level groups as follows:

Dosage Levels (ppm)	Number of Mice	
	Male	Female
0 (Control)	75	75
50	75	75
250	75	75
1000	75	75

Five mice/sex were also maintained at each dosage level and in the control group for 4 weeks as possible replacement animals. One control male (missing) and one high-dose female (sacrificed *in extremis*) were replaced during this 4-week period; therefore, 76 female animals were available to be examined pathologically at the high dose level. At the end of the 4-week period, all replacement mice not used on study were sacrificed and appropriately discarded.

The mice were housed individually in suspended wire-mesh cages and maintained in a temperature-, humidity-, and light-controlled room. The light cycle was 12-hr light/12-hr dark. Values and ranges were not reported for temperature and humidity. Control and test diets were prepared using Purina Laboratory Chow® #5001 or Certified Rodent Chow® #5002 (beginning in June, 1979, the twelfth month of the study), and water were available *ad libitum*.

Certification analysis of each lot of Certified Rodent Chow® #5002 (Ralston Purina Company, St. Louis, Missouri) was performed by Raltech Scientific Services, St. Louis, Missouri. Beginning in 1979, the International Research and Development Corporation (IRDC) water supply was analyzed quarterly for the presence of heavy metals, pesticides, and coliform bacteria.

The mice were ear punched to indicate the treatment level. Ear punches were verified at each cage change and before necropsy.

This study was initiated on July 7, 1978. A one-year interim sacrifice of 10 mice/sex/group was conducted on July 6, 1979. The study was terminated by sacrifice of approximately one-third of each group/day on July 7, 8, and 9, 1980.

Test Article Administration

Orthene Technical (RE-12420) was offered in the diet at dosage levels of 50, 250, and 1000 ppm. The test diets were prepared as follows: on the day before the diet preparation, the frozen test compound was removed from cold storage and allowed to reach room temperature; the appropriate amount of the test compound was then weighed in a beaker, dissolved in distilled water and added to 500 g of the basal laboratory feed; additional distilled water, used to rinse the beaker, was also added. A Hobart food mixer was used for mixing (10 minutes). The resulting premix was then blended in a twin-shell blender with an additional amount of basal laboratory feed

for 30 minutes (the intensifier bar was run for 2 minutes at the beginning and end of the 30-minute period) to yield prepared test diets at dosage levels of 50, 250, and 1000 ppm. Due to an increase in the total amount of diet being prepared, the premix was increased to 1000 grams of basal laboratory chow during the time period of 3/9/79 - 8/10/79. For the control diet, distilled water (equal in quantity to the treated diets) was mixed with basal laboratory feed.

Fresh diets were prepared on day 0 of each study week. Approximately one-half of the prepared diet was administered at the beginning of the test week (day 0). Unoffered test diets were frozen and stored. On day 4 of each study week, additional (thawed) test diet was offered. On September 24, 1978 (week 11) and December 1, 1978 (week 21) the test diets were inadvertently maintained overnight at room temperature.

General Observations

1. Appearance and Behavior

The mice were observed three times daily (Monday through Friday) or twice daily (weekends and holidays) for signs of overt toxicity. These signs were recorded on the day they were noted. Detailed observations including tissue masses were recorded weekly.

2. Mortality

Moribundity and mortality were recorded on the day observed.

3. Body Weights

Body weights were recorded weekly for the first 8 weeks of the study and monthly thereafter.

4. Food and Compound Consumption

Individual food consumption was recorded weekly for the first 8 weeks of study and monthly thereafter. From these values, the compound consumption values were calculated using the nominal concentration of Orthene Technical in the diet.

Hematology

Hematologic studies were conducted on blood samples from 10 mice/sex/group at termination of the study. Blood was collected via puncture of the orbital sinus plexus.

Hematological determinations included hemoglobin[1], hematocrit[1], erythrocyte count[1], total leucocyte count[1], platelet count[1], mean corpuscular volume (MCV)[1], mean corpuscular hemoglobin (MCH)[1], mean corpuscular hemoglobin concentration (MCHC)[1], reticulocyte count[2], and differential leucocyte count[2].

Most hematologic parameters were measured on the Ortho ELD-8 [1]; this instrument automatically calculates and reads out values for mean

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1 Ortho ELT-8 Operators Manual, Ortho Instruments, Westwood, MA., 1979.

2 Miale, J.B., Laboratory Medicine-Hematology, 5th ed., The C.V. Mosby Company, 1977.

corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Pathology

After 12 months of dietary administration of the test article, 10 mice per sex per group were sacrificed by carbon dioxide asphyxiation and were subjected to complete postmortem examinations. After 24 months all surviving mice were similarly sacrificed and examined, and all animals dying during the course of the study or sacrificed *in extremis* were examined as soon as possible after death.

Postmortem examinations were performed under the direct supervision of a pathologist and consisted of a thorough evaluation for external abnormalities including palpable masses and an inspection of orifices. The skin was then reflected from a ventral midline incision and any subcutaneous masses were identified and correlated with antemortem findings. The organs of the abdominal, thoracic, and cranial cavities were examined *in situ* and after removal and dissection. Any morphologic changes observed were recorded on the Pathology Record Sheet.

A number of animals were necropsied at the terminal sacrifice before authorization to determine organ weights was received from the sponsor. The following organs from terminally sacrificed animals were trimmed free of fat and connective tissue and weighed:

brain with stem
heart
liver
gonads
kidneys

Hematoxylin and eosin stained paraffin sections of the following tissues were prepared by standard histologic methods and examined microscopically from all mice from all groups which were sacrificed at termination or which died or were sacrificed in extremis.

aorta
adrenals (2)
sternum (bone, bone marrow)
brain (2 sections)
ears (middle)
esophagus
eyes, Harderian glands
gallbladder
testes/epididymides or ovaries
heart
small intestine (duodenum jejunum, ileum)
large intestine (cecum, colon)
kidneys (2)
liver
lung (inflated with formalin), mainstem bronchi
lymph nodes (mesenteric, mediatinal)
mammary gland
nasal cavity, paranasal sinuses
sciatic nerve
pancreas
pituitary
prostate or uterus (corpus, cervix, and horns)
salivary glands
seminal vesicles
skeletal muscle
skin
spinal cord (2 levels)
spleen
stomach
thymus
trachea
thyroid
urinary bladder
gross changes of uncertain nature, tissue masses, or suspect tumors and regional lymph nodes
blood smear (if anemia, enlarged thymus, lymphadenopathy or hepatosplenomegaly was present)

Tissues were trimmed and processed histologically by personnel of International Research Development Corporation (IRDC). All tissues were examined microscopically by IRDC pathologists and a consultant pathologist.

Statistics

All statistical analyses compared the treatment groups with the control group by sex.

Body weights (weeks 13, 26, 39, 52, 65, 78, 91, and 104), food consumption (weeks 0-13, 17-39, 43-65, 69-78, 82-91, and 95-104), hematological parameters (terminal), and absolute and relative organ weights (terminal), were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances, and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie[1] and Ostle[2]. Dunnett's[3] multiple comparison tables were used to judge significance of differences.

Randomization Procedure

In order to assign animals to test groups, animal numbers and the corresponding body weights were entered onto magnetic tape which was used as the data source for the following randomization procedure. First, the mean body weight and standard deviation were calculated by sex, and a computer-generated edit developed a listing of those animals whose body weights were within ±1.5 standard deviations of the mean. From these qualifying animals, the randomization procedure selected and assigned the required number of

1 Steel, R.G.D. and Torrie, J.H. (1960), Principles and Procedures of Statistics, McGraw-Hill, New York, N.Y.
2 Ostle, Bernard, Statistics in Research, Iowa State College Press, 1954.
3 Dunnett, C.W., (1964), New Tables for Multiple Comparisons with a Control, Biometrics 20:482-491.

animals. Bartlett's Chi-square test for homogeneity of variances as described by Steel and Torrie[1] was applied to the groups. If the groups were not judged to be homogeneous, new randomizations were applied until homogeneity was established.

Diet Analysis

Triplicate 50 g samples of the control and test diets (prepared on day 0 of each study week) were taken as follows: weeks 1-3 (day 0 and 7), weeks 4-12 (day 0, 4, and 7) and weeks 17, 21, 25, 30, 34, 36, 38, 43, 47, 51, 56, 60, 65, 69, 73, 82, 86, 91, 95, 99, and 104 (day 0, 4, and 7). Also, triplicate samples were taken on days 0, 3, and 7 for week 78.

Two samples were shipped frozen to the sponsor for test material analysis. One sample was stored frozen at IRDC.

At week 6, 10 g of the day 7 test diet sample for the 50-ppm dosage levels was inadvertently added to the day 7 sample for the 250-ppm dosage level. This sample was shipped to the sponsor and analyzed for test material content.

At week 13 day 4, five samples collected from feeders placed in an empty cage and five samples collected from pooled diets of feeders in cages containing mice were sent to the sponsor. In addition, on week 22, five day-0 samples, five day-4 samples from feeders in empty

[1] Steel, R.G.D. and Torrie, J.H. (1960); Principles and Procedures of Statistics, McGraw-Hill, New York, N.Y.

cages, and five day-4 samples from feeders in cages containing mice were sent to the sponsor to assess any influence of the mice on the degradation of the test compound.

9. Results and Discussion

The lifetime oral carcinogenicity study in mice is stated to have been conducted according to the protocol specified by the sponsor. The protocol was not included in the report (in addition, the original protocol must not have included the determination of organ weights as a toxicological end-point since authorization for organ weight determinations was received on day one of the terminal sacrifice). Justificiation of species and strain selection was not given other than historical experience at the testing facility.

Chemical analyses of the test article, Orthene Technical, SX-1032, determined the concentration of acephate to be 92.7% on 6/28/78, 92.4% on 9/27/79, and 92.3% on 1/15/80. Thus, the acephate solution was stable throughout the experimental period.

No explanation was given for the selection of the dose levels. Oncogenicity and chronic toxicity studies usually require that the high dose be the MTD.

The specific facility of Charles River Breeding Laboratories, Inc. from which the mice were delivered was not reported. Information about the facility may be important because the mouse assessment profile (viral profile) is different for each facility and

may help explain the etiology of the unusual lung lesions described in the report.

It appears that the animals were identified by ear-punch as to treatment group and not by individual animal numbers. Animal numbers were apparently attached to the cage and it was the cage numbers that were randomized. It is impossible to verify that the pathology sheet or body weights for a given animal number are for the same animal associated with that cage number at randomization other than to know by ear-punch that the animal was from the same treatment group.

The initial randomization of animals is well described but no indication was given as to the method of selection or sequence of processing of the animals for the interim sacrifice and for each day of the final sacrifice or for the selection of animals for hematological evaluation.

Two animals were replaced during the first 4-weeks of the study.

The environmental conditions including excursions in temperature and relative humidity were not reported.

Diet Analyses

The grand average of the analytical data for all batches and the variation between batches are given in Table 1. While the grand averages and percent of nominal concentration results are acceptable values, the coefficient of variation is large. The range

TABLE 1

SUMMARY OF DIET ANALYSES

Nominal Concentrations		Results of Diet Analyses			
Orthene Technical (ppm)	Acephate in Orthene Technical (ppm)	Sample Type*	Grand Average (ppm Acephate)	Percent of Nominal Concentration of Acephate	Coefficient of Variation (%)
50	46.4	1 2 3	45.2 40.3 39.4	97.4 86.9 84.9	15 29 13
250	232	1 2 3	226 241 227	97.4 103.9 97.8	12 15 21
1000	927	1 2 3	879 879 839	94.8 94.8 90.5	17 13 14

*Sample type = 1 - Freshly prepared diet
2 - Diet from cages after 4 days
3 - Diet from cages after 7 days (stored) frozen for 4 days prior to use)

of analytical values for each sample type are presented in Table 2. An average of 30% of all samples are outside of the range of ±20% of the nominal concentration. With this type of variation, one might suspect problems with homogeneity, stability and/or actual preparation of the diet mixture. The use of the internal standard and spike samples when diet samples were analyzed insured accurate analytical data. Stability and homogeneity studies were not reported. Stability problems can arise from temperature and/or relative humidity excursions. No data were presented in this study to evaluate possible involvement of temperature and relative humidity in the stability of acephate in the diet. Thus, one might question the preparation or stability of the diet and, since not all samples were analyzed (one of every 4 preparations after week 8), one cannot be certain as to exact treatment levels.

While none of the 50 ppm analytical values overlapped any of the 250 ppm samples, several of the 250 ppm samples were higher than some of the 1000 ppm samples. On at least one occasion (week 38), the 50 ppm rats may have been fed the 1000 ppm diet and vice versa. This could also have occurred (unobserved) in other weeks, since diet analyses were performed on only one of every four preparations. It should be noted that the procedure for mixing the diet was changed on several occasions without explanation of the reasons for the change.

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TABLE 2

VARIATION IN DIET ANALYSES

Nominal Concentrations		Results of Diet Analyses		
Orthene Technical (ppm)	Nominal Concentration of Acephate in Orthene Technical (ppm)	Sample Type*	Range of Analytical Values (ppm)	Percent of Samples Outside the Range of the Nominal ± 20%
50	46.4	1 2 3	21.8 - 65.0 21.0 - 75.0 28.0 - 61.0	26 45 42
250	232	1 2 3	141 - 484 160 - 385 100 - 345	22 26 34
1000	927	1 2 3	378 - 1400 450 - 1310 414 - 1830	22 26 31

*Sample type = 1 - Freshly prepared diet
2 - Diet from cages after 4 days
3 - Diet from cages after 7 days (stored) frozen for 4 days prior to use)

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Mortality

As indicated in Table 3, more male mice in the 250 ppm and 1000 ppm groups survived to terminal sacrifice (47 and 46, respectively) than in the 0 ppm and 50 ppm groups (33 and 37, respectively). The differences between groups were marginally significant (Chi-square = 7.37, D.F. = 3, $p \leq 0.06$). Fewer females in the 50 ppm and 250 ppm (25 and 26, respectively) survived to terminal sacrifice than in the 0 ppm and 1000 ppm (31 and 34, respectively). This difference was not significant (Chi-square = 4.36, D.F. = 3, $p \leq 0.22$).

Body Weight

Body weight data for male mice are given in Table 4 and for female mice in Table 5. Statistical significance of body weight changes was determined for selected weeks (every 13 weeks) but standard deviations were not reported. Male and female mice in the 1000 ppm group did not gain weight during the first four weeks of the study. The 1000 ppm mice exhibited 8-30% lower body weights than controls during the study. The authors attributed the "appearance" of labored breathing to body weight loss during weeks 41-71 in the treated mice. It is clear from Tables 4 and 5 that no substantative changes in body weight were noted after week 30. On occasion, e.g.,

TABLE 3

NUMBER OF ANIMALS DYING, MISSING OR SACRIFICED AND NUMBER OF ANIMALS EXAMINED DURING STUDY PERIOD

Dose and Segment	Number Dying, Sacrificed, or Missing		Number Examined for Histopathology	
	M	F	M	F
0 ppm				
0-52 wks	3[a]	1	2	1
Interim sac	10	10	10	10
53-105 wks	30	33	30	33
Final sac	33	31	33 (22)	31 (21)
TOTAL	76	75	75	75
50 ppm				
0-52 wks	3	4	3	4
Interim sac	10	10	10	10
53-105 wks	25	36	25	36
Final sac	37	25	37 (24)	25 (16)
TOTAL	75	75	75	75
250 ppm				
0-52 wks	0	2	0	2
Interim sac	10	10	10	10
53-105 wks	18	37[a]	18	36
Final sac	47	26	47 (31)	26 (17)
TOTAL	75	75	75	74
1000 ppm				
0-52 wks	3	5	3	5
Interim sac	10	10	10	10
53-105 wks	16	27	16	27
Final sac	46	34	46 (30)	34 (22)
TOTAL	75	76	75	76

[a]One animal missing.
Number in parenthesis is the number of animals whose organs were weighed.

TABLE 4

SELECTED GROUP MEAN AND RANGE OF BODY WEIGHTS FOR MALE MICE
(grams)

Study Week	0 ppm	50 ppm	250 ppm	1000 ppm	Percent of Control (1000 ppm Group)
0	24(22-26)	25(22-26)	24(22-26)	24(22-26)	100
1	26(22-30)	27(23-30)	26(22-29)	24(21-28)	92
2	28(25-34)	29(23-32)	28(22-32)	24(21-28)	86
3	30(20-37)	29(24-33)	29(22-33)	24(20-29)	80
4	29(23-35)	30(25-34)	30(22-34)	25(22-29)	86
13[a]	34(27-41)	34(29-40)	34(27-40)	29(22-34)[c]	85
26[a]	36(24-44)	35(18-45)	34(28-42)[c]	29(24-35)[c]	81
30	37(24-46)	37(31-48)	36(29-41)	30(24-36)	81
34	37(29-48)	36(31-49)	35(29-42)	30(24-35)	81
36	37(28-48)	36(30-49)	35(29-41)	30(25-34)	81
39[a]	37(29-49)	37(32-52)	36(30-44)[b]	30(25-35)[c]	81
43	37(30-49)	37(32-51)	36(29-44)	29(22-34)	78
47	37(31-48)	36(31-50)	34(28-42)	28(23-33)	76
52[a]	40(29-51)	38(25-55)	37(30-44)[c]	30(22-31)[c]	75
65[a]	40(30-52)	39(32-53)	36(28-44)[c]	29(24-37)[c]	73
78[a]	40(32-51)	37(29-51)[c]	36(29-44)[c]	28(22-34)[c]	70
91[a]	39(30-50)	37(31-46)	35(25-44)[c]	29(21-36)[c]	74
104[a]	38(27-53)	37(29-46)	34(47-65)[c]	29(20-34)[c]	76

[a]Weeks when the statistical significance of differences between treatment and control groups were tested (t-test).
[b]Significantly different than Control Group Mean, $p \leq 0.05$.
[c]Very significantly different than Control Group Mean, $p \leq 0.01$.

TABLE 5

SELECTED GROUP MEAN AND RANGE OF BODY WEIGHTS FOR FEMALE MICE
(grams)

Study Week	0 ppm	50 ppm	250 ppm	1000 ppm	Percent of Control (1000 ppm Group)
0	20(18-22)	20(18-22)	20(18-22)	20(18-22)	100
1	22(18-25)	22(19-26)	22(19-25)	20(17-23)	91
2	23(19-27)	24(21-28)	23(19-26)	20(12-24)	87
3	24(20-29)	25(21-29)	24(18-29)	20(17-25)	83
4	25(20-29)	25(21-29)	25(20-29)	21(18-25)	84
13[a]	28(24-34)	29(23-34)	29(22-35)	24(19-29)[c]	86
26[a]	30(26-38)	31(24-38)	30(22-37)	24(18-28)[c]	80
30	32(27-40)	32(26-40)	32(23-39)	25(19-30)	78
34	32(27-38)	32(26-42)	31(25-39)	25(20-34)	78
36	31(26-39)	32(27-41)	31(25-38)	25(19-34)	81
39[a]	32(27-39)	32(26-40)	31(24-38)	26(20-37)[c]	81
43	32(25-41)	32(25-41)	31(23-39)	25(19-34)	78
47	31(24-41)	32(25-41)	30(21-37)	24(19-30)	77
52[a]	33(26-44)	34(27-44)	31(23-40)[b]	25(20-32)[c]	76
65[a]	33(25-43)	33(27-43)	31(23-38)[c]	25(29-33)[c]	76
78[a]	33(28-42)	33(26-41)	30(22-38)[c]	25(19-31)[c]	76
91[a]	34(23-42)	32(22-42)	30(24-41)[c]	25(17-31)[c]	74
104[a]	35(24-48)	33(21-41)	30(22-36)[c]	25(18-30)[c]	71

[a]Weeks where the statistical significance of differences between treatment and control groups were tested (t-test).
[b]Significantly different than Control Group Mean, $p \leq 0.05$.
[c]Very significantly different than Control Group Mean, $p \leq 0.01$.

week 65 for male mice and week 78 for female mice, the 250 ppm animals weighed 10% less than control animals. Based on body weights as reported in this study, it would appear that the MTD for CD-1 mice may be less than 1000 ppm of Orthene Technical. It should be noted that an additional body weight measurement was recorded on March 16, 1979 following a change in the diet mixing and feeding procedure.

Food Consumption

Food consumption values were not reported for individual mice. It is not clear if individual food consumption values were recorded. Group mean values are reported in terms of g/mouse/day and g/kg/day for several weeks of the study. From these values and by using the "nominal concentration" of Orthene Technical in the diet, mg/kg/day were calculated. Food consumption data for males are given in Table 6 and for females in Table 7. The decrease in food consumption with increasing concentration of Orthene Technical indicate possible problems with palatability. For statistical analyses, average mean values for each group were calculated for each 13 week period. Data that appear unusual are the food consumption values for week 43 for both male (Table 6) and female mice (Table 7). There was an across-the-board decrease of approximately 30% in food consumption for all groups at week 43 which returns to previous levels at week 47. Values were not recorded for the surrounding weeks (weeks 40,

TABLE 6

MALES: MEAN FOOD AND COMPOUND CONSUMPTION

Week of Study	0 ppm (Control)		50 ppm			250 ppm			1000 ppm		
	Food		Food		Compound	Food		Compound	Food		Compound
	g/ mouse/ day	g/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day
0	5.1	212.2	5.1	211.1	11	5.3	222.5	56	5.1	210.7	211
1	5.9	226.5	5.5	205.1	10	5.0	190.5	48	5.1	213.7	214
2	6.7	238.0	6.2	215.4	11	5.3	190.5	48	3.9	164.1	164
3	5.1	169.7	4.4	151.8	8	5.1	176.2	44	3.6	149.8	150
4	4.1	141.7	4.3	143.1	7	4.9	163.2	41	3.5	166.8	141
5	6.1	202.1	5.9	196.2	10	5.3	175.6	44	4.2	160.6	161
6	6.0	186.0	6.4	199.8	10	5.7	177.3	44	4.4	167.8	168
7	5.3	171.7	6.3	202.4	10	5.3	169.8	42	4.6	176.3	176
8	5.2	161.2	5.7	183.0	9	5.5	178.4	45	3.8[a]	140.9[a]	141[a]
13	4.8	146.8	4.2	122.1	6	4.4	128.2	32	3.8	131.8	132
17	5.4	159.6	5.6	165.9	8	5.5	167.7	42	4.4	158.6	159
21	4.8	135.8	5.1	149.0	7	4.3	125.1	31	3.8[b]	130.3[b]	130[b]
26	4.5	124.3	4.2	119.2	6	4.0	116.5	29	3.5[c]	122.1[c]	122[c]
30	5.1	138.1	5.2	239.9	7	4.5	124.6	31	4.0	133.3	133
34	4.7	126.7	4.9	135.3	7	5.1	144.8	36	4.6	153.2	153
36	4.7	127.7	4.7	129.4	6	4.9	140.5	35	4.0	133.1	133
39	4.9	133.4	5.0	135.8	7	4.4	122.6	31	4.2	138.7	139
43	3.6	96.4	3.6	96.8	5	3.1	86.5	22	3.0	103.7	104
47	4.5	123.0	4.8	132.9	7	4.5	132.0	33	4.0	143.8	144
52	4.8	119.9	4.9	129.6	6	4.7	126.5	32	4.3	143.7	144
56	4.6	115.3	4.7	124.2	6	4.3	120.5	30	4.2	150.7	151
61	4.7	117.9	4.8	123.7	6	4.5	126.1	32	4.3	142.1	142
65	5.0	124.1	4.9	126.2	6	4.7	130.1	33	4.5	153.9	154

TABLE 6

(CONCLUDED)

Week of Study	0 ppm (Control)		50 ppm			250 ppm			1000 ppm		
	Food		Food		Compound	Food		Compound	Food		Compound
	g/ mouse/ day	g/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day
69	4.8	121.1	4.9	129.2	6	4.6	127.0	32	4.7	161.0	161
74	4.9	124.9	4.9	131.7	7	4.7	129.9	32	4.6	163.3	163
78	4.9	122.9	4.8	130.4	7	4.7	130.9	33	4.5	159.1	159
82	4.8	126.2	4.7	128.0	6	4.6	128.1	32	4.3	149.6	150
87	4.8	123.5	4.7	129.9	6	4.6	130.2	33	4.2	151.0	151
91	4.6[d]	118.6[d]	4.8	129.7	6	4.5[e]	128.7[e]	32[e]	4.1	139.8	140
95	5.0	127.4	5.0	135.4	7	4.6	135.1	34	4.0	144.3	144
100	4.8	125.8	4.8	130.6	7	4.4	130.3	33	4.2	148.9	149
104	4.7	124.2	4.8	130.6	7	4.6	135.7	34	4.1	142.2	142

[a]Data for one mouse not recorded.
[b]Data for two mice not included due to urine soaked diet.
[c]Data for one mouse missing due to urine soaked diet.
[d]Data for one mouse missing due to broken food jar.
[e]Data for one mouse not recorded.

TABLE 7

FEMALES: MEAN FOOD AND COMPOUND CONSUMPTION

Week of Study	0 ppm (Control)		50 ppm			250 ppm			1000 ppm		
	Food		Food		Compound	Food		Compound	Food		Compound
	g/ mouse/ day	g/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day
0	4.5	224.2	4.6	232.4	5	4.5	223.3	56	4.5	227.0	227
1	4.4	202.3	4.2	189.5	9	4.9	221.6	55	4.7	236.2	236
2	5.5	238.6	5.5	230.6	12	5.0	218.5	55	3.5	173.3	173
3	5.4	226.0	5.3	211.6	11	5.5	228.7	57	3.3	163.7	104
4	3.8	152.2	3.7[a]	148.2[a]	7[a]	3.7	149.5	37	2.9	136.8	137
5	5.2[a]	207.6[a]	4.8[b]	185.2[b]	9[b]	4.9	189.7	47	4.6[a]	207.4[a]	207[a]
6	5.7	217.9	6.2[a]	227.9[a]	11[a]	6.1[b]	235.6[b]	59[b]	3.8	174.7	175
7	6.0[b]	223.2[b]	6.9[c]	254.3[c]	13[c]	5.8[b]	214.3[b]	54[b]	5.1[a]	229.7[a]	230[a]
8	5.6	215.3	5.7	212.7	11	5.2	200.6	50	4.0	180.0	180
13	5.0	177.1	5.0	171.0	9	4.7	171.0	40	3.5	147.9	148
17	6.0	207.5	6.1	120.4	11	5.8	199.5	50	4.3	178.8	179
21	5.2	179.6	5.1	168.5	8	4.8	165.1	41	3.2[e]	133.4[e]	133[e]
26	5.2	174.1	5.1	165.9	8	4.8[d]	160.6[d]	40[d]	3.8	156.9	157
30	5.9	183.5	5.5	171.1	9	4.8	149.9	37	4.8	153.1	153
34	5.2	163.9	5.4	168.5	8	5.5	177.3	44	4.7	188.7	189
36	5.8	188.1	5.4	169.9	8	4.9	158.8	40	4.0	160.5	160
43	3.8	117.5	3.6	113.9	6	3.4	109.6	27	2.9	118.0	118
47	4.9	157.5	5.1	154.4	8	4.4	147.4	37	4.0	166.8	167
52	5.0	152.7	5.2	152.4	8	4.6	147.8	37	4.2	168.1	168
56	5.2	163.7	5.1	154.5	8	4.4	145.9	36	4.3	171.3	171
61	5.0	146.2	5.0	146.4	7	4.6	149.8	37	4.3	164.8	165
65	5.2	156.4	5.0	150.3	8	4.7	150.7	38	4.3	172.6	173

TABLE 7

(CONCLUDED)

Week of Study	0 ppm (Control)		50 ppm			250 ppm			1000 ppm		
	Food		Food		Compound	Food		Compound	Food		Compound
	g/ mouse/ day	g/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day	g/ mouse/ day	g/ kg/ day	mg/ kg/ day
69	5.0	148.0	5.1	153.9	8	4.6	154.7	39	3.8	150.2	150
74	4.9	147.7	5.0	150.6	8	4.7	157.8	39	4.6	183.5	183
78	4.8	146.4	5.0	151.2	8	4.7	156.5	39	4.2	168.9	169
82	4.7	143.4	4.7	147.5	7	4.5	146.6	37	4.2	159.8	160
87	4.7	142.5	4.6	145.0	7	4.3	142.9	36	3.8	157.7	158
91	4.6	134.5	4.7	145.7	6	4.3	144.9	36	3.8	153.8	154
95	4.7	137.2	4.8	150.1	8	4.2	140.5	35	3.8	156.4	156
100	4.8	138.3	4.7	147.9	7	4.0	134.3	34	3.6	145.6	146
104	5.0	142.2	5.1	153.1	8	4.3	144.4	36	3.6	144.6	145

[a]Data for one mouse not recorded.
[b]Data for two mice not included due to urine soaked diet.
[c]Data for one mouse missing due to urine soaked diet.
[d]Data for one mouse missing due to broken food jar.
[e]Data for one mouse not recorded.

41, 42, 44, 45, or 46) so that one cannot determine if problems existed in these weeks. This may have implications in the labored breathing reported between weeks 41-71 and the eventual gross and microscopic lung lesions seen in all groups of mice including the controls. An abrupt decrease in food consumption was also recorded for week 4 for all groups of female and most groups of male mice. The most common situations that can lead to a decrease in food consumption are: (1) a bacterial or viral infection; (2) lack of water; (3) stress due to elevated temperature; (4) stress due to changes in relative humidity; (5) lack of food; (6) change in personnel or procedures in the daily care of the animals; and 7) general cleanliness of the room and/or cages. It is not possible to determine if any of these possibilities led to a reduction in food consumption. It should be noted that an additional food consumption measurement period was conducted on March 9-16, 1979 following a change in the diet mixing and feeding procedure, the details of which were not given.

Mean values for food and compound consumption by dose group and sex (based on nominal concentration) are given in Table 8. No treatment-related changes in food consumption were noted except as possibly related to palatability. The reduced food consumption resulted in smaller mice in the treated groups. Females received slightly higher doses of the test article (mg/kg/day) than males.

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TABLE 8

AVERAGE FOOD AND COMPOUND CONSUMPTION

Dosage Level (ppm)	Average Food Consumption (g/kg/day)		Average Food Consumption (g/kg/day)		Average Compound Consumption (mg/kg/day)	
	Male	Female	Male	Female	Male	Females
0	5.1	5.1	144	172	0	0
50	5.0	5.1	146	160	7	8
250	4.7	4.7	144	168	36	42
1000	4.2	4.0	146	167	146	167

* CONFIDENTIAL BUSINESS INFORMATION *

Appearance and Behavior

Individual data for the clinical health of the mice were not reported. Incidental findings noted for control and treated mice included hair loss, scabbing, corneal opacity, red and/or swollen eyelids, lacrimation, distended, swollen or firm abdomen, yellow stained fur, pale or blue exposed skin, labored breathing, masses, and signs of moribundity. Of these, only labored breathing appeared to be treatment related, according to the authors. Incidences of the clinical signs noted in the study were not given. The authors stated that the clinical signs were not treatment-related. An incidence table would be required to verify the statement that no treatment-related effects were seen.

In reviewing the data for labored breathing, the authors concluded that the labored breathing, which appeared to be dose-related during weeks 41-71, was an artifact of body weight loss. MITRE cannot agree with this conclusion based on the data reported. No unusual changes in body weight were evident after week 30. Therefore, there is no reason to attribute the reported labored breathing to body weight loss between weeks 41-71. When one examines the food consumption data for week 43, it is apparent that something unusual happened in that all groups of animals consumed approximately 30% less food that week as compared to weeks 30-39 or week 47.

Whatever occurred affected both control and treated mice. Since the 250 ppm and 1000 ppm groups may have been stressed more than the control or 50 ppm group, signs of clinical health such as labored breathing may have been more pronounced for the 250 ppm and 1000 ppm mice. In addition, the change in food consumption and the reported labored breathing may be related to the unusual lung lesions present in control and treated mice in this study.

Hematology

Hematological determinations were made only at the terminal sacrifice. Certain of the hematological values for 40% of the 1000 ppm males and for 100% of the 1000 ppm females for which determinations were made were considered invalid due to an equipment malfunction. These data should not have been lost since additional animals could have been bled on day 2 or day 3 during the 3 day sacrifice. Loss of the samples precludes a complete evaluation of the hematological effects. Of the values reported, no data were remarkable even though some data points were statistically significantly different from control values as seen in Table 9 for male mice and Table 10 for female mice.

Organ Weights

A large number of animals were necropsied on the first day of the terminal sacrifice prior to receipt of authorization to evaluate

TABLE 9

MALES: MEANS, STANDARD DEVIATION, N, AND SIGNIFICANCE OF HEMATOLOGICAL VALUES

Hematology	Study Month	0 ppm (Control)			50 ppm			250 ppm			1000 ppm		
		$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N
Erythrocyte[a] $10^6/mm^3$	24	7.59	0.953	10	7.31	1.013	10	8.54[b]	0.406	10	8.36[b]	0.354	6[c]
Leucocytes $10^3/mm^3$	24	7.2	3.75	10	5.4	1.91	10	5.4	2.01	10	5.2	1.54	6[c]
Neutrophils (Seg.) /100 WBC	24	47	14.7	10	50	16.0	10	48	10.3	10	52	15.0	10
Lymphocytes /100 WBC	24	45	15.5	10	41	16.8	10	41	9.8	10	35	14.6	10
Hematocrit %	24	41.8	7.26	10	39.2	6.53	10	48.0	4.13	10	48.5	3.93	6[c]
Hemoglobin g/dl	24	14.1	2.64	10	12.7	2.09	10	15.6	1.18	10	15.5	1.36	10
Platelet[a] $10^3/mm^3$	24	996	9.2	10	994	21.6	10	981	34.8	10	911	101.1	6[c]

TABLE 9

(CONCLUDED)

Hematology	Study Month	0 ppm (Control)			50 ppm			250 ppm			1000 ppm		
		$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N
Reticulocyte /100 WBC	24	5.6	1.57	10	4.8	3.00	10	4.3	1.27	10	3.9[b]	0.88	10
MCV μm^3	24	55	3.6	10	53	2.8	10	56	3.2	10	58	2.6	6[c]
MCH pg	24	18.4	1.55	10	17.2[b]	0.79	10	18.3	0.81	10	18.7	0.73	6[c]
MCHC %	24	33.6	0.85	10	32.3[d]	0.51	10	32.6	0.74	10	32.4[d]	0.71	6[c]

[a] These means include values with > signs. See individual animal data for the number of such values included.
[b] Significantly different from Control group mean, $p \leq 0.05$.
[c] Four values considered invalid were not included in statistical analysis.
[d] Significantly different from Control group mean, $p \leq 0.01$.

TABLE 10

FEMALES: MEANS, STANDARD DEVIATION, N, AND SIGNIFICANCE[a] OF HEMATOLOGICAL VALUES

Hematology	Study Month	0 ppm (Control)			50 ppm			250 ppm			1000 ppm		
		$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N
Erythrocyte[b] $10^6/mm^3$	24	7.40	1.263	10	7.79	0.598	10	7.70	1.239	10	-[c]	-	-
Leucocytes $10^3/mm^3$	24	9.2	10.43	10	5.9	2.26	10	8.8	5.29	10	-[c]	-	-
Neutrophils (Seg.) /100 WBC	24	47	11.5	10	40	12.4	10	42	14.1	10	48	10.8	10
Lymphocytes /100 WBC	24	35	10.7	10	52	10.4	10	48	13.7	10	43	10.2	10
Hematocrit %	24	41.9	6.76	10	44.2	5.96	10	44.1	8.29	10	-[c]	10.2	10
Hemoglobin g/dl	24	13.7	2.40	10	14.4	1.98	10	14.5	2.78	10	16.0	11.75	10
Platelet[b] $10^3/mm^3$	24	883	180.4	10	860	105.0	10	897	185.4	10	-[c]	-	-

TABLE 10

(CONCLUDED)

Hematology	Study Month	0 ppm (Control)			50 ppm			250 ppm			1000 ppm		
		$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N	$\bar{X}$	S.D.	N
Reticulocyte /100 WBC	24	5.8	4.90	10	4.5	1.54	10	7.8	7.73	10	5.3	1.96	10
MCV (μm^3)	24	57	6.6	10	57	3.8	10	57	4.6	10	-[c]	-	-
MCH pg	24	18.7	2.18	10	18.4	1.23	10	18.8	1.50	10	-[c]	-	-
MCHC %	24	32.7	0.79	10	32.3	0.77	10	32.8	0.68	10	-[c]	-	-

[a]No statistical significance found.
[b]These means include values with > signs. See individual animal data for the number of such values included.
[c]Values considered invalid not included in statistical analysis.

organ weight as a toxicological end-point (one would have thought organ weight determination would have been specified in the protocol).

The authors reported a statistically significant decrease in absolute brain weight in the 1000 ppm females. Changes in brain weight are unusual and the occurrence of such changes is an indication that the 1000 ppm dose level may be above the MTD. The authors also reported an increase in the relative brain weights in the treated animals as shown in Table 11. The large increase in relative liver weight in the 1000 ppm female mice is due to the inclusion of four animals with livers weighing more than 5.0 grams rather than the expected 1.5-3.0 grams. These four animals had proliferative liver changes, i.e., hepatocellular carcinoma. The other animals in the group that had proliferative liver changes had livers weighing less than 3 grams. When these four animals are deleted, the absolute and relative liver weights for the female mice in the 1000 ppm group are 1.75 g and 7.28%, respectively.

Pathology

Inspection of the pathology data leads one to question the care with which the study was conducted. In the authors' table (Table 10 of the report), they do not list any hyperplastic nodules in the liver of male mice at the interim sacrifice, while another pathologist at the same laboratory (Table 12 of the report) listed

TABLE 11

ABSOLUTE (GRAMS) AND RELATIVE (% BODY WEIGHT) ORGAN WEIGHTS

Group Sex	Body Wt.	Liver		Kidneys		Heart		Brain		Testes (g) Ovaries	Testes %X10 Ovaries
	(g)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(mg)	(%X10)
0 ppm											
M	36	2.37	6.54	0.86	2.39	0.25	0.71	0.49	1.36	0.22	5.89
F	34	2.24	6.49	0.62	1.83	0.24	0.70	0.50	1.50	415	11.87
50 ppm											
M	36	2.20	6.08	0.83	2.32	0.26	0.71	0.50	1.40	0.21	5.91
F	33	2.06	6.44	0.62	1.92	0.23	0.70	0.49	1.51	430	13.05
250 ppm											
M	34	2.09	6.22	0.77[a]	2.32	0.28	0.84	0.49	1.47	0.23	7.01
F	30	1.85	6.23	0.51[b]	1.73	0.24	0.80	0.48	1.61	376	12.05
1000 ppm											
M	28	1.75[b]	6.22	0.69[b]	2.48	0.23	0.81	0.49	1.77[b]	0.23	8.44[b]
F	25	2.52	9.90[a]	0.46[b]	1.85	0.22	0.89	0.46[b]	1.88[b]	101[b]	3.85[b]

[a]Significantly different from 0 ppm group ($p \leq 0.05$).
[b]Very significantly different from 0 ppm group ($p \leq 0.01$).

one hyperplastic nodule in the liver of one male mouse in the 50 ppm group. However, the pathology data in the appendix (Appendix E of the report) lists one hyperplastic nodule in the liver of one male mouse in both the 50 and 250 ppm groups. Since the liver pathology data is vital to the study, it is important to know which pathologist's observations are given for deaths, unscheduled sacrifices, and scheduled sacrifices, since a real difference exists in their diagnoses. These types of differences can lead to real inconsistencies within the data. However, in reviewing the incidences of liver lesions during the second year of study and the final sacrifice, there appears to be good agreement between listed incidences and the individual pathological observations given in the appendix (Appendix E of the report).

Remarkable pathology observations for this study were primarily limited to the lung and the liver.

Respiratory tract lung lesions consisted of pigmented macrophages often associated with eosinophilic crystalloid bodies, alveolar hyalinosis, and acute rhinitis. Since fibrosis and other signs of chronic injury were not present, the authors considered the lung lesions to be reversible.

An incidence table of selected respiratory tract lesions is given in Table 12. Several lesions are included because of differences in the description of lesions used by the several pathologists involved in this study. There is a definite progression in severity with time and treatment for the non-neoplastic lesions and a clear dose-response for the presence of macrophages. These data indicate that the LEL for Orthene Technical would be 50 ppm while the NOEL was not determined in this study. The eosinophilic foreign bodies were unusual and had never been seen in control mice by any of the pathologists involved with the study. They were always associated with the dark-pigmented macrophages. The authors considered these non-neoplastic lesions to be treatment-related rather than a predisposition due to genetics, shipping, and/or care of the animals. Since these eosinophilic foreign bodies had never been seen by the pathologists in control animals before, their presence may indicate that the control animals received some test article. This could happen if: 1) Orthene Technical was volatile at the temperature and relative humidity experienced in this study; 2) Orthene Technical recrystallized as minute crystals in the diet which could become airborne as the mice scurried about in their feed dish and cage; and/or 3) the diet between control and treated mice was switched on one or more ocassions. Although the lung lesions were

TABLE 12

INCIDENCE OF SELECTED RESPIRATORY TRACT LESIONS[a]

Site and Lesion	0 ppm						50 ppm						250 ppm						1000 ppm					
	I[b]		U[c]		T[d]		I		U		T		I		U		T		I		U		T	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number Examined	10	10	32	34	33	31	10	10	28	40	37	25	10	10	18	38	47	26	10	10	19	32	46	34
LUNG																								
- Foam cell foci	0	0	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
- Brownish pigmented alveolar macrophages	0	0	1	0	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
- Macrophage accumulations	1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	8	7	0	0	0	0
- Dark pigmented alveolar macrophages	0	0	3	0	3	8	2	0	8	18	8	17	2	4	12	34	42	25	0	0	14	24	46	34
- Eosinophilic foreign bodies	0	0	0	0	3	3	0	0	0	1	3	3	0	0	1	2	5	3	0	0	2	4	13	11
- Alveolar hyalinosis	2	1	0	1	0	1	1	0	0	5	2	2	0	0	2	5	3	3	0	0	0	0	11	16
- Adenocarcinoma[e]	0	0	0	0	2	1	0	0	0	0	2	2	0	0	0	0	2	0	0	0	0	0	1	1
- Adenoma[e]	0	0	1	0	11	9	0	0	0	0	10	5	0	0	0	0	13	3	0	0	0	0	11	5
- Carcinoma[e]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
NASAL CAVITY																								
- Acute rhinitis	0	0	3	3	0	0	0	0	2	6	1	2	0	0	3	4	11	6	0	0	6	8	14	11
- Adenocarcinoma[e]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

[a]Only positive findings are listed.
[b]Interim Sacrifice.
[c]Unscheduled Deaths and Sacrifices.
[d]Terminal Sacrifice.
[e]Incidence data for neoplastic lesions are for the entire study.

unusual, they probably do not have much bearing on the carcinogenicity of Orthene Technical. Based on these lung changes, the LEL for Orthene Technical is 50 ppm and the NOEL was not determined.

Treatment related changes in the liver consisted of mononuclear cell foci, hepatocyte hypertrophy, karyomegaly, and intranuclear inclusion bodies in both male and female mice and, in addition, hyperplastic nodules and hepatocellular carcinoma in the female mice.

An incidence table of selected liver lesions is given in Table 13. It is clear the Orthene Technical is a liver toxin and that 50 ppm is the NOEL while 250 ppm is the LEL for liver lesions. A closer inspection of the data for hyperplastic nodules, hepatocellular adenomas, and hepatocellular carcinomas and their corresponding gross lesions is given in Table 14. The incidence of these lesions over segments of the study period is given in Table 15. The fact that only 2 hepatocellular carcinomas (in control male mice) were noted prior to the final necropsy and the total absence of a tumor response in the 250 ppm female mice are quite remarkable.

Of all reported neoplastic lesions, only 9 tumor types were notably different among the test groups. These are given in Table 16. There are reduced numbers of total neoplasms at 250 ppm and 1000 ppm for male mice and 250 ppm female mice as compared to

TABLE 13

INCIDENCE OF SELECTED LIVER LESIONS

Liver Lesion Type - Lesion	0 ppm						50 ppm						250 ppm						1000 ppm					
	I[a]		U[b]		T[c]		I		U		T		I		U		T		I		U		T	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number Examined	10	10	32	34	33	31	10	10	28	40	37	25	10	10	18	38	47	26	10	10	19	32	46	34
NON-NEOPLASTIC																								
- Mononuclear Cell Infiltrates/Foci[d]	4	4	1	2	8	17	4	3	3	7	16	19	3	0	3	6	16	17	6	3	11	20	31	28
- Hepatocyte Hypertropy	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	9	10	10	9	10	15	45	30
- Karyomegaly	0	0	0	0	0	0	0	0	4	0	0	0	0	0	3	2	14	10	0	0	6	14	37	24
- Intranuclear Inclusion Bodies	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	11	4	0	0	0	12	44	19
- Hyperplastic Nodules	0	0	3	1	7	1	1	0	1	0	5	1	1	0	0	0	3	0	0	0	4	4	9	11
NEOPLASTIC																								
- Hepatocellular Adenoma	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	2	0	1
- Hepatocellular Carcinoma	0	0	2	0	2	1	0	0	0	0	2	1	0	0	0	0	3	0	0	0	0	0	3	12

[a]Interim Sacrifice
[b]Unscheduled Deaths and Sacrifices
[c]Terminal Sacrifice
[d]Pathology data for Interim sacrifice recorded by different pathologists.

TABLE 14

SELECTED LIVER LESIONS ASSOCIATED WITH GROSS NECROPSY FINDINGS

Gross Lesions - Microscopic Lesions	0 ppm		50 ppm		250 ppm		1000 ppm	
	M	F	M	F	M	F	M	F
Total Examined	75	75	75	75	75	74	75	76
CYST (TOTAL REPORTED)[a]	(5)	(2)	(2)	(5)	(2)	(9)	(1)	(4)
- Cyst	3	1	2	2	1	5	--	2
- Hyperplastic Nodule	1	--	--	--	--	--	--	1
- No Cyst Evident	1	--	--	--	--	--	--	--
- Hemangiosarcoma	--	--	--	--	--	--	1	--
- No Lesion Listed	--	1	--	3	1	2	--	1
NODULE (TOTAL REPORTED)	(3)	(3)	(1)	(--)	(1)	(--)	(4)	(3)
- Hyperplastic Nodule	3	1	1	--	--	--	3	2
- No Lesion Listed	--	2	--	--	--	--	1	--
- Hepatocellular	--	--	--	--	--	--	--	1
MASS (TOTAL REPORTED)	(9)	(4)	(11)	(4)	(7)	(1)	(13)	(26)
- Hyperplastic Nodule	5	2	6	--	3	--	9	10
- Hepatocellular Adenoma	--	--	1	2	1	--	1	3
- Hepatocellular Carcinoma	4	1	2	1	3	--	3	12
- Hemangiosarcoma	--	1	2	--	--	--	--	1
- No Lesions Listed	--	--	--	1	--	1	--	--
NO CORRESPONDING GROSS LIVER LESION (TOTAL FOUND)	(2)	(--)	(--)	(1)	(1)	(1)	(1)	(3)
- Cyst	1	--	--	--	--	1	--	--
- Hyperplastic Nodule	1	--	--	1	1	--	1	2

[a]The total reported refers to the listing given under gross lesions in the individual pathology data, while the diagnoses are the corresponding description given in the corresponding microscopic lesions section of the pathology table.

TABLE 15

INCIDENCE OF SELECTED LIVER LESIONS AT VARIOUS SEGMENTS OF THE STUDY PERIOD

Study Period - Liver Lesion	0 ppm		50 ppm		250 ppm		1000 ppm	
	M	F	M	F	M	F	M	F
0-52 WEEKS	(2)[a]	(1)	(3)	(4)	(0)	(2)	(3)	(5)
- Hyperplastic Nodules	--	--	--	--	--	--	--	--
- Hepatocellular Adenoma	--	--	--	--	--	--	--	--
- Hepatocellular Carcinoma	--	--	--	--	--	--	--	--
INTERIM SACRIFICE	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
- Hyperplastic Nodules	--	--	1	--	1	--	--	--
- Hepatocellular Adenoma	--	--	--	--	--	--	--	--
- Hepatocellular Carcinoma	--	--	--	--	--	--	--	--
53-78 WEEKS	(5)	(12)	(7)	(6)	(6)	(5)	(4)	(9)
- Hyperplastic Nodules	1	1	--	--	--	--	--	--
- Hepatocellular Adenoma	--	--	--	--	--	--	--	--
- Hepatocellular Carcinoma	--	--	--	--	--	--	--	--
79-91 WEEKS	(10)	(4)	(12)	(11)	(4)	(6)	(2)	(9)
- Hyperplastic Nodules	1	--	1	--	--	--	--	2
- Hepatocellular Adenoma	--	--	--	--	--	--	--	--
- Hepatocellular Carcinoma	1	--	--	--	--	--	--	--

TABLE 15

(CONCLUDED)

Study Period - Liver Lesion	0 ppm		50 ppm		250 ppm		1000 ppm	
	M	F	M	F	M	F	M	F
92-105 WEEKS	(15)	(17)	(6)	(19)	(8)	(25)	(10)	(9)
- Hyperplastic Nodules	1	--	--	--	--	--	4	1
- Hepatocellular Adenoma	--	--	1	1	--	--	1	2
- Hepatocellular Carcinoma	1	--	--	--	--	--	--	--
TERMINAL SACRIFICE	(33)	(31)	(37)	(25)	(47)	(26)	(46)	(34)
- Hyperplastic Nodules	7	1	5	1	3	--	9	11
- Hepatocellular Adenoma	--	--	--	1	1	--	--	1
- Hepatocellular Carcinoma	2	1	2	1	2	--	3	12

[a]Number in parentheses is number of animals examined during the study period segment

TABLE 16

INCIDENCE OF SELECTED NEOPLASTIC MICROSCOPIC LESIONS

Site - Neoplastic Lesion	0 ppm		50 ppm		250 ppm		1000 ppm	
	M	F	M	F	M	F	M	F
Total Examined	75	75	75	75	75	74	75	76
HARDARIAN GLAND								
- Adenoma	10	5	8	1	8	0	2	1
HEMATOPOIETIC LYMPHOCYTIC, RETICULO-ENDOTHELIAL SYSTEMS								
- Malignant Lymphoma	3	11	2	8	2	3	2	3
- Reticulum Cell Sarcoma	3	4	2	5	0	6	0	0
LIVER								
- Hepatocellular Carcinoma	4	1	2	1	3	0	3	12
- Hemangiosarcoma	0	1	2	1	0	0	1	1
- Hepatocellular Adenoma	0	0	1	2	1	0	1	3
- Hemangioma	0	0	0	0	0	0	0	1
SPLEEN								
- Hemangioma	1	0	1	0	0	0	3	2
- Hemangiosarcoma	0	0	0	0	0	1	2	1
TOTAL NEOPLASMS	21	22	18	18	14	10	14	24
% INCIDENCE	28	29	24	24	19	14	19	32

controls. The 32% neoplasms in female mice at 1000 ppm is not significantly different from 29% in control female mice. Thus, what can one say about the high incidence of hepatocellular carcinomas in female mice in the 1000 ppm group? Three approaches to that question are as follows:

Historical control data - Often historical control data are useful in identifying an unusual low tumor incidence in the control group on study. Historical control data for CD-1 female mice among control animals were obtained and, for hepatocellular carcinomas, the incidence ranged from 0 to 6% in 22 studies at the same testing facilities. When compared to the 15.8% incidence observed in female mice in the 1000 ppm group, these historical data would indicate that the incidence is significantly different from control values.

Maximum tolerated dose - According to the NCI definition, "the maximum tolerated (MTD) dose should be the highest dose that causes no more than a 10% weight decrement, as compared to the appropriate control groups; and does not produce mortality, ..." (NCI Carcinogenesis Technical Report Series No. 1, February 1976, "Guidelines For Carcinogen Bioassay in Small Rodents," NCI-CG-TR-1, p. 15). Using this definition, the 1000 ppm level exceeded the MTD since the animals in this group experienced a 30 percent weight decrement. Inspection of the body data indicate that the 250 ppm group experienced a 10% weight decrement and could thus qualify as the MTD. If this were the case, Orthene Technical would not be considered a carcinogen based on the fact that the 250 ppm animals exhibited a reduced number of neoplasms as compared to control animals.

Fatal and Incidental Tumors - Peto, et al. in their article entitled, "Guidelines For Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments," (International Agency for Research on Cancer Monograph Series, Supplement 2, pp. 311-426) state that tumors found at scheduled sacrifices are incidental tumors

as compared to those tumors found in animals dying on study or animals sacrificed in extremis which are fatal or mortality-independent tumors depending on the cause of death. Without going into great detail of this approach, all 12 of the heptocellular carcinomas found in the female mice of the 1000 ppm group were present at the terminal sacrifice and would be classified as incidental tumors. Using this approach, Orthene Technical would not be considered to increase the onset of cancer based on the longevity of the female mice with hepatocellular carcinomas.

Based on these two approaches, Orthene Technical could be considered a potential carcinogen but not a proven carcinogen. Based on this study, Orthene Technical (1000 ppm - highest dose tested) was carcinogenic in female mice only at the terminal sacrifice (hepatocellular carcinoma).

The 1000 ppm male mice had an increased incidence of multifocal intratubular mineralization in the renal cortex of the kidney which was considered to be treatment-related.

Thus, Orthene Technical is a liver and lung toxicant in male and female CD-1 mice.

10. Technical Review Time: 195 hours.