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

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FEB 2 - 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Difenzoquat Methyl Sulfate (Technical AC 84,777): 83-1 Chronic Feeding Study in the Nonrodent and 83-2 Carcinogenicity Study in Mice.

DP Barcode No.: D193941 Submission No.: S445822
EPA Id No.: 106401 P.C. Code No.: 106401
Rereg. Case No.: 0223 Case No.: 819395
CAS No.: 43222-48-6 Tox. Chem. No.: 363 A

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C)
Krystyna K. Locke 1/19/94

TO: Walter Waldrop, PM Team No. 71
Reregistration Branch
Special Review and Reregistration Division (7508W)
KB 1/25/94

THRU: Roger Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C)
Roger Gardner 1/25/94

Section I, Toxicology Branch I/HED had completed an evaluation of the following studies:

83-1 One-Year Oral Toxicity Study in Purebred Beagle Dogs with AC 84,777 via Capsule Administration; Catherine M. Kelly; Bio/dynamics, Inc., East Millstone, NJ; Report No.: 90-3640; Study Completion Date: May 6, 1993.
MRID No.: 42800401

83-2 Chronic Dietary Toxicity and Oncogenicity Study with AC 84,777 in Mice; Karen M. MacKenzie; Hazleton Laboratories America, Inc., Madison, WIS.; Report No.: HLA 6123-145; Study Completion Date: June 23, 1989.
MRID No.: 42800402

The above studies were submitted in response to the requirements of the 1988 Registration Standard for Difenzoquat Methyl Sulfate.

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In the dog study, the animals received Difenzoquat in capsules at the following dose levels (mg/kg/day): 0 (Group I), 12.5 (Group II), 37.5/20 (Group III), 75/50/44/30 (Group IV) and 125/100/75 (Group V). Group II was dosed at the 12.5 mg/kg level from day 1 through week 52. Groups III and IV were dosed, respectively, with 37.5 and 75/50/44 mg/kg Difenzoquat during days 1-28, and with 20 and 30 mg/kg, respectively, from day 29 through week 52. The dose levels in these groups were decreased due to poor or no food consumption, poor health and/or high mortality. All dogs in Group V died by day 9 and this group was terminated. Severe toxic signs (detailed in the Tox. Branch review of this study) were observed only at Difenzoquat levels of 44-125 mg/kg/day and a decreased food consumption was noted at the 37.5 mg/kg level. The only toxic sign observed at the 30 mg/kg level of Difenzoquat was a decreased body weight gain throughout the study in the female dogs. NOEL and LOEL were, therefore, as follows:

Systemic NOEL: 20 mg/kg/day; females
30 mg/kg/day; males

Systemic LOEL: 30 mg/kg/day; females (decreased body weight gains)

Classification: Core-Guideline

In the mouse study, the animals were administered Difenzoquat for 79/80 weeks at dietary levels of 0, 200, 500 and 1000 ppm. These values corresponded to 0, 26.9, 69.4 and and 150,1 mg/kg/day for males and 0, 39.7, 97.9 and 202.4 mg/kg/day for females (mean achieved doses of Difenzoquat). NOEL and LOEL were as follows:

Systemic NOEL: 200 ppm (26.9 mg/kg/day); males
500 ppm (97.9 mg/kg/day); females

Systemic LOEL: 500 ppm (69.4 mg/kg/day); males
[decreased body weight gains]

1000 ppm (202.4 mg/kg/day); females
[decreased body weight gains]

Classification: Core-Guideline

Difenzoquat was not carcinogenic in this study.

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Primary Review by: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (7509C) Krystyna K. Locke 1/19/94

Secondary Review by: Roger Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (7509C) Roger Gardner 1/25/94

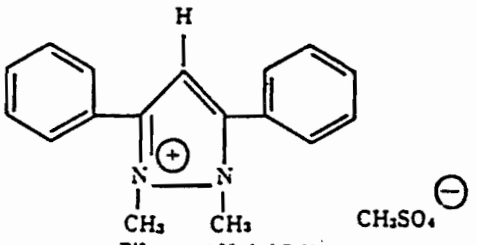
DATA EVALUATION RECORD

STUDY TYPE: 83-1 Chronic Feeding in the Nonrodent

EPA IDENTIFICATION NUMBERS:

MRID No.: 42800401	DP Barcode No.: D193941
EPA ID No.: 106401	Submission No.: S445822
Case No.: 819395	Rereg. Case No.: 0223
P.C. Code No.: 106401	Tox. Chem. No.: 363 A

TEST MATERIAL: Technical AC 84,777 (Difenzoquat methyl sulfate; Avenge; 1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium methyl sulfate; selective herbicide); Chem. Abs. Registry No.: 43222-48-6; white to off-white free flowing powder, received at the testing facility on January 29, 1991; Supplier: the sponsor; Lot No.: AC 6027 -118; Purity: 99.4%; stable for 5 years or longer when stored in an airtight container in a dark, dry place at room temperature; expiration date: February 1996. Structure:



Difenzoquat Methyl Sulfate

REPORT NUMBER: 90-3640

SPONSOR: American Cyanamid Company, Princeton, New Jersey.

TESTING FACILITY: Bio/dynamics, Inc., East Millstone, New Jersey

TITLE OF REPORT: One-Year Oral Toxicity Study in Purebred Beagle Dogs with AC 84,777 via Capsule Administration.

AUTHOR: Catherine M. Kelly

STUDY COMPLETION DATE: May 6, 1993

SUMMARY

Groups of purebred beagle dogs (4-6/sex/dose) were administered Difenzoquat in capsules for 52 weeks. Group I (controls) received empty gelatin capsules and Group II, 12.5 mg/kg/day of Difenzoquat. The remaining groups were dosed with Difenzoquat as follows: Group III - 37.5 mg/kg/day for the first 28 days and 20 mg/kg from day 29 through week 52; Group IV - 75 mg/kg for the first 6 days, 50 mg/kg during days 7 and 8, 44 mg/kg during days 9-28 and 30 mg/kg from day 29 through week 52; and Group V - 125 mg/kg for the first 4 days, 100 mg/kg during days 5 and 6, and 75 mg/kg until day 9 when all dogs died or were sacrificed moribund. It was not reported how the initial doses were selected. However, due to mortality, poor health and lack of food cosnumption observed in Groups IV and V, doses for these groups were decreased until the dogs could tolerate them. Due to continued poor food consumption in Group III, Difenzoquat level was also decreased in that group. Therefore, the final dose levels received by the treated dogs in Groups II through IV, from day 29 (week 5) until study termination (week 52) were 12.5, 20 and 30 mg/kg/day, respectively. Dosing was performed 7 days/week.

Toxic signs were not observed in male and female dogs which were dosed with Difenzoquat at levels of 12.5 and 20 mg/kg/day. There were also no toxic signs in male dogs receiving 30 mg/kg/day of Difenzoquat. However, female dogs dosed with 30 mg/kg/day of Difenzoquat gained significantly ($p = 0.05$ of 0.01) less weight than did the controls (40-64% of the conrtol values) throughout the study. No other toxic sings were observed.

Toxic signs were observed mostly at Difenzoquat levels of 44 -125 mg/kg/day, which were used during the first 28 days of the study, and included:

1. High mortality - 4 dogs (1/6 males and 3/6 females) died in Group IV and all dogs (4/4 males and 4/4 females) died in Group V.

2. Watery and/or tarry stools, emesis, salivation, tremors, lethargy, irregular gait, lateral recumbency, dilated pupils, and partially or completely closed eyes.

3. Loss of weight during weeks 1-2 and decreased weight gain (by 38% for males and 89% for females) during weeks 3-4, relative to the control values.

4. Decreased food consumption for Group IV males (25-36%) and females (35-50%), and for Group V males (54-99.5%) and females (50-99.5%), relative to the control values.

5. Macroscopic findings in the nonsurviving dogs: discoloration and/or abnormal contents of the esophagus, stomach,

and small and large intestines.

6. Microscopic findings in the nonsurviving dogs: myocardial degeneration (6 dogs), myocardial necrosis (1 dog) and lesions in the gastrointestinal tract: necrotic ulcer and acute inflammation of the esophagus; luminal or mucoid exudate, necrotic ulcer and congestion of the stomach; and congestion, exudate and necrotic ulcer in the large and small intestines.

Systemic NOEL: 20 mg/kg/day; females
30 mg/kg/day; males

Systemic LOEL: 30 mg/kg/day (decreased body weight gains); females

Classification: Core-Guideline

EXPERIMENTAL PROCEDURES:

Dosing via gelatin capsules was initiated on March 6, 1991 and was terminated on March 4, 5 or 8, 1992 (after 365-369 days). The dogs were sacrificed and necropsied on March 5, 6 and 9, 1992, respectively.

This study was originally a dietary study in which dogs were fed diets containing 500, 1500, 3000 or 5000 ppm of Difenzoquat. However, after 2 weeks of poor food consumption, the route of Difenzoquat administration was changed to gelatin capsules. Appropriate amounts of Difenzoquat, adjusted on the basis of each dog's most recent body weight, were weighed and placed into gelatin capsules daily. Control animals received empty gelatin capsules. Records were maintained of the amount of Difenzoquat placed in each gelatin capsule.

At study initiation, the dogs received the following dose levels of Difenzoquat (mg/kg/day): 0 (control group; Group I), 12.5 (Group II), 37.5 (Group III), 75 (Group IV) and 125 (Group V). However, due to mortality, poor health and lack of food consumption observed in Groups IV and V, dose levels were decreased as follows: for Group IV, from 75 mg/kg to 50 mg/kg on day 7, to 44 mg/kg on day 9, and to 30 mg/kg on day 29; and for Group V, from 125 mg/kg to 100 mg/kg on day 5 and to 75 mg/kg on day 7. On study day 9, the surviving dogs in Group V were euthanized due to continued poor health. Also, due to continued poor food consumption in Group III, Difenzoquat was decreased in this group from 37.5 mg/kg to 20 mg/kg on day 29. **Therefore, the final dose levels received by the treated dogs in Groups II through IV, from week 5 (day 29) until study termination (week 52), were 12.5, 20 and 30 mg/kg/day, respectively.** Dosing was performed 7 days per week at the time when food was removed.

At study initiation, there were 6 dogs of each sex in the control group, and 4 dogs of each sex in each of the Difenzoquat-treated Groups II through V. However, starting with study day 15 (March 20, 1991), Group IV also had 6 dogs of each sex (at the sponsor's request, 2 male and 2 female dogs were added to this group on that date). These 4 dogs were treated with Difenzoquat through March 17, 1992 and were sacrificed on March 18, 1992 (week 54). It was not reported how the initial dose levels were selected in this study. **The dogs were:**

(1) Obtained from Marshall Farms, U.S.A., Inc., North Rose, New York.

(2) Acclimated for approximately 2 months (from January 3 through March 5, 1991).

(3) Assigned to groups as follows: Dogs were first ranked by body weight and distributed into 5 groups of 4 or 6

animals per sex so that the body weight means for each group were comparable. Groups were then assigned randomly to dose levels.

(4) About 6 months old and weighed 7.0-8.8 kg (mean: 7.8 kg; males) and 4.4-8.3 kg (mean: 6.0 kg; females) at the start of the treatment.

(5) Housed individually in elevated metal grid cages, at temperatures of 66-76°F, relative humidity of 26-95% and 12 hours light/12 hours dark cycles.

(6) Identified by ear tattoo numbers and numbered ear tags, and by cage and group numbers.

(7) Allowed 400 g of standard laboratory diet (Purina Certified Canine Diet #5007) for one hour daily during study days 1 through 9. To improve food consumption, the dogs were given 300 g of standard laboratory diet and 100 g of Kal Kan Pedigree and/or Alpo daily for 1 hour during study days 10-12. Food presentation was increased to 4 hours daily from study day 13 through termination. During the first month, Kal Kan Pedigree, Alpo and baby food were given overnight to some dogs as a supplement to increase food (standard laboratory diet) consumption. Also, to increase their food consumption, vitamin B complex was given to one Group II female dog during study days 53-56 and to one Group III male dog during study days 43-50.

The following parameters were examined for all dogs on the study unless indicated otherwise:

(1) Observations: At least once daily, approximately 2 hours after dosing, for mortality and gross signs of toxic effects.

(2) Physical Examinations: Detailed, prior to initiating treatment and weekly thereafter.

(3) Ophthalmoscopic Examinations: In the week before the start of treatment, during months 3 and 6, and at study termination. Lids, lacrimal apparatus and conjunctiva were examined grossly; cornea, anterior chamber, lens, iris, vitreous humor, retina and optic disc were examined by indirect ophthalmoscopy.

(4) Body Weight: Before initiation of treatment, weekly during treatment and terminally (after fasting).

(5) Food Consumption: Daily, before and during treatment.

(6) **Hematology and Clinical Chemistry:** In the week before before the start of treatment, during week 6, during months 3 and 6, and at study termination. Performed on all surviving animals at the intervals indicated. Blood was obtained by jugular venipuncture before feeding and dosing. The following determinations were performed:

Hematology:

White blood cell and differential white cell count
Red blood cell and platelet count
Hemoglobin and hematocrit

The following values were calculated:

Mean corpuscular hemoglobin
Mean corpuscular hemoblobin concentration
Mean corpuscular volume

Clinical Chemistry:

Aspartate aminotransferase	Albumin
Alanine aminotransferase	Globulin (calculated)
Alkaline phosphatase	A/G ratio (calculated)
Lactic dehydrogenase	Total bilirubin
Creatine phosphokinase	Direct bilirubin
Gamma glutamyl transpeptidase	Sodium
Blood urea nitrogen	Potassium
Creatinine	Chloride
Fasting glucose	Calcium
Cholesterol	Inorganic phosphorus
Total protein	

(7) **Urinalysis:** In the week before the start of treatment, during week 6, during months 3 and 6, and at study termination. Performed on all surviving animals at the intervals indicated. Urine was collected in stainless steel metabolism pans attached to each animal's permanent cage. Dogs were not deprived of food prior to urine collections. The following determinations were performed:

Gross appearance	Bilirubin
Specific gravity	Occult blood
pH	Urobilinogen
Protein	16-Hour volume
Glucose	Microscopic analysis
Ketones	

(8) **Necropsy:** Performed on all dogs in this study, including those found dead or killed in a moribund condition. Animals were fasted prior to scheduled sacrifices by exsanguination under sodium pentobarbital anesthesia. The necropsy included a macroscopic examination of the external surface of the body; all orifices; the carcass; the external surfaces of the brain and spinal cord; the cranial, thoracic, abdominal and pelvic cavities, and their viscera; and the cervical tissues and organs.

(9) **Organ Weights:** At the terminal sacrifice; the following organs were weighed: brain, kidneys, liver, ovaries, spleen, testes and thyroid/parathyroid glands. Paired organs were weighed together. Relative weights (organ/body weight and organ/brain weight ratios) were calculated.

(10) **Histopathology:** The following tissues were examined for all dogs on the study:

Brain (3 sections including medulla/pons and cerebellar and cerebral cortices)

Intestine (cecum, colon, duodenum, ileum, jejunum and rectum)

Lungs (with mainstem bronchi)
Lymph nodes (mesenteric and submaxillary)
Mammary gland (female and male)
Spinal cord (cervical, thoracic and lumbar)
Sternum and femur with bone marrow
Uterus (corpus and cervix)
Gross lesions (including a section of normal-appearing portion of same tissue)

Adrenals
Aorta (thoracic)
Epididymides
Esophagus
Eyes
Gall bladder
Heart
Kidneys
Liver
Nerve (sciatic)
Optic nerve
Ovaries
Oviducts
Pancreas
Parathyroid glands
Pituitary gland
Prostate gland
Salivary glands
Skeletal muscle
Skin (abdominal)
Spleen
Stomach
Testes
Thymus
Thyroid gland
Trachea
Urinary bladder
Vagina
Tissue masses

The above tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. Before examination, eyes were placed in Zenker's solution for the initial 24 hours, washed overnight in running water and then trasferred to 70% Synosol®. Testes and epididymides were first fixed in Bouin's solution, then in 70% Synosol® and finally in formalin. All other tissues were preserved in 10% neutral buffered formalin.

(11) Dose Level Confirmation: One random sample was taken from the weekly supply of capsules of each dog, from each dose group, at weeks 1, 2, 3, 4, 8, 13, 17, 21, 26, 30, 34, 39, 43, 47 and 52. These samples, along with the calculation of the amount of Difenzoquat in the capsules for each dose level, were then sent immediately under room temperature conditions to the sponsor for analysis. The expected levels of Difenzoquat (mg/kg) and the corresponding weekly dog weight were also submitted to the sponsor. Capsule contents were determined by weighing contents on an analytical balance and by HPLC analysis.

Statistical Analyses:

Mean body weights, weekly changes in mean body weights, body weight changes from week 0, food consumption, hematology, clinical chemistry, terminal body weights and terminal organ weights (both absolute and relative) were analyzed statistically as described in Attachment I of this review. In general, the above data were first evaluated by one-way analysis of variance (ANOVA) using F-test for variance comparison. If significant differences anong the means were indicated, then Dunnett's t-test at the 95% confidence level was used to determine which means were significantly different from the controls.

RESULTS

Dose Level Confirmation

Diets fed initially to dogs for 2 weeks averaged 100-102% of nominal concentrations of Difenzoquat, with coefficients of variation of 3-5%. The nominal concentrations of Difenzoquat were 500, 1500, 3000 and 5000 ppm.

Capsules were properly filled with Difenzoquat. Capsule contents for the low-dose group (12.5 mg/kg of body weight), mid-dose group (37.5/20 mg/kg) and high-dose group (75/50/44/30 mg/kg) averaged 99, 99 and 100%, respectively, of nominal concentration of Difenzoquat. The overall coefficient of variation for each group was 1%. Capsule contents for the 125/100/75 mg/kg group, which was terminated after 9 days due to high mortality,

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averaged 100% of nominal. The gravimetric and HPLC results of capsule contents were similar.

Clinical Findings

Clinical findings, attributed to treatment with Difenzoquat, were observed only during the first 2 weeks of the study in Groups IV and V, at dose levels ranging from 44 to 125 mg/kg/day. These findings were watery and/or tarry stools, emesis, salivation, tremors, lethargy, irregular gait, lateral recumbency, dilated pupils, partially or completely closed eyes and little or no food consumption. Treatment-related clinical findings were not observed at Difenzoquat doses of 12.5, 20 and 30 mg/kg/day. The 12.5 mg/kg dose was administered to dogs for 52 weeks and the 20 and 30 mg/kg doses, for about 48 weeks (from day 29 to termination.

Mortality

Difenzoquat-related mortality was observed only in Group IV (75/50/44/30 mg/kg/day) and in Group V (125/100/75 mg/kg/day) at dose levels ranging from 44 to 125 mg/kg/day. In Group IV, 1 male and 3 females were found dead after 13-17 days of treatment. In Group V, 3 males and 3 females were found dead during study days 4-9 and the remaining 2 dogs (male and female) were sacrificed moribund on study day 9. No mortality was observed in Group IV after Difenzoquat was decreased to 30 mg/kg/day on day 29. No mortality was also observed in Group I (controls), Group II (12.5 mg/kg/day) and Group III (37.5/20 mg/kg/day). The mortality data are summarized below.

Group	I	II	III	IV	V
	Number of dogs assigned to study				
Males	6	4	4	6	4
Females	6	4	4	6	4
	Number of dogs killed or found dead				
Terminal sacrifice					
Males	6	4	4	5	0
Females	6	4	4	3	0
Unscheduled deaths					
Males	0	0	0	1	4
Females	0	0	0	3	4

Continued on next page

Group	I	II	III	IV	V
	Number of dogs killed or found dead				
Found dead					
Males	0	0	0	1	3
Females	0	0	0	3	3
Moribund sacrifice					
Males	0	0	0	0	1
Females	0	0	0	0	1

This table is based on Appendix B (Animal Termination History), page 63 of the submitted report (MRID No. 42800401).

Unscheduled Deaths - Male Dogs

Group mg/kg/day ■	Day of dose initiation	Number of dogs on test	Test days 1	4	5	9	13
I							
0	1	6	0	0	0	0	D
II							
12.5	1	4	0	0	0	0	D
III							
37.5	1	4	0	0	0	0	-
IV							
75	1	6	0	0	0	-	-
50	7	6	-	-	-	0	-
44	9	6	-	-	-	0	D
V							
125	1	4	0	1D	-	-	-
100	5	3	-	-	1D	-	-
75	7	2	-	-	-	1D 1M	-

This table is based on Appendix B (Mortality Summary: Test Days 1 to 28 - Males), page 59 of the submitted report (MRID No. 42800401).

■ Difenzoquat

D = Found dead M = Sacrificed moribund

Unscheduled Deaths - Female Dogs

Group mg/kg/day ■	Day of dose initiation	Number of dogs on test	Test days 1	4	8	9	14	17	27
I									
0	1	6	0	0	0	0	0	0	0
II									
12.5	1	4	0	0	0	0	0	0	0
III									
37.5	1	4	0	0	0	0	0	0	0
IV									
75	1	6	0	0	-	-	-	-	-
50	7	6	-	-	0	-	-	-	-
44	9	6	-	-	-	0	1D	1D	1D ‡
V									
125	1	4	0	1D	-	-	-	-	-
100	5	3	-	-	-	-	-	-	-
75	7	3	-	-	1D	1D 1M	-	-	-

This table is based on Appendix B (Mortality Summary: Test Days 1 to 28 - Females), page 61 of the submitted report (MRID No. 42800401).

■ Difenzoquat D = Found dead M = Sacrificed moribund

‡ At the sponsor's request, 2 dogs were added to Group IV on test day 15. One of these dogs died on test day 27, meaning that it was exposed to Difenzoquat for 13 days.

Ophthalmoscopic Examination

Treatment-related ocular abnormalities were not observed. During the pretest examination, nuclear Y lens opacities were observed in 1 male dog and small optic disks were observed in 4 other dogs (1 male and 3 females). These conditions persisted throughout the study. During the month 6 examination, iritis was noted in 1 high-dose (30 mg/kg) female and, at the termination of the study, distichiasis was noted in another high-dose female. However, Lionel F. Rubin, V.M.D. (Bryn Mawr, PA), who examined the dogs during the course of this study, did not attribute these last two findings to treatment with Difenzoquat.

Body Weights

These data were reported as (1) weekly mean and individual body weights; (2) weekly mean and individual body weight changes; and (3) weekly mean and individual body weight changes from week 0 (start of treatment) values.

Mean body weight gains were significantly lower than concurrent control values in Group V (125/100/75 mg/kg/day) which was terminated after 9 days of treatment. Relative to the concurrent control values, mean body weight gains were also significantly lower in Group IV, in males during week 2 (at dose level of 44 mg/kg/day) and in females during weeks 2-4 (at dose level of 44 mg/kg/day) and during weeks 5, 6, 22, 26, 27, 33, 43, 46-48 and 52 (at dose level of 30 mg/kg/day). Difenzoquat had no effect on body weight gains of male and female dogs in Groups II (12.5 mg/kg/day) and III (37.5/20 mg/kg/day), and male dogs in Group IV (30 mg/kg/day). The body weight changes observed in this study are summarized below.

Mean Body Weight Gains of Male Dogs

Group	I	II	III	IV	V
Week	Body weight gains (kg)				
1	0.0	0.2	0.1	- 0.2	- 0.6
2	0.2	0.4	0.5	- 0.3 *	T
4	0.8	0.9	0.8	0.5	
5	0.8	1.0	0.8	0.8	
10	1.7	2.0	2.1	1.7	
20	3.0	2.9	3.5	3.0	
30	3.4	3.1	3.9	3.5	
40	3.5	3.7	4.1	4.0	
50	4.1	4.1	4.3	4.3	
52	4.2	4.2	4.5	4.3	

This table is based on Appendix G (Mean Body Weight Change from Week 0 Values - Males), pages 248-254 of the submitted report (MRID No. 42800401).

* Statistically significant difference from the control group mean at the 5% level (Dunnett's t-test).

T = Terminated on day 9.

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Mean Body Weight Gains of Female Dogs

Group	I	II	III	IV	V
Week	Body weight gains (kg)				
1	0.1	0.0	0.0	- 0.4	- 0.6 *
2	0.5	0.4	0.3	- 0.8 **	T
3	0.8	0.8	0.7	0.0 **	
4	0.9	0.9	0.8	0.1 **	
5	1.0	1.0	1.1	0.4 *	
6	1.3	1.2	1.3	0.6 **	
12	2.1	1.6	2.4	1.5	
22	3.5	3.7	3.7	2.2 *	
26	4.0	4.0	3.9	2.4 *	
27	3.9	3.9	4.1	2.5 *	
33	4.2	4.2	4.1	2.2 *	
43	4.7	5.2	4.7	2.7 *	
46	4.8	5.0	4.9	2.7 *	
47	4.8	5.1	4.8	2.5 *	
48	4.8	5.3	5.0	2.8 *	
50	5.0	5.4	5.0	3.0	
52	5.4	5.8	5.1	3.0 *	

This table is based on Appendix G (Mean Body Weight Change from Week 0 Values - Females), pages 255-261 of the submitted report (MRID No. 24800401).

* and ** Statistically significant difference from the control group mean at the 5% and 1% level, respectively (Dunnett's test).

T = Terminated on day 9.

Food Consumption

These data were reported (1) as daily mean food consumption (g/group) for weeks 1 through 4; and (2) as weekly mean and individual food consumption (g/kg/day) for weeks 0 through 52 or 54 (for the four dogs added to Group IV on day 15). Difenzoquat decreased food consumption only at dose levels ranging from 44 to 125 mg/kg/day.

Male and female dogs in Group V (125/100/75 mg Difenzoquat/kg of body weight/day) consumed significantly less food than did the controls during study days 2 through 5 and then stopped eating. By day 9, all dogs in this group either died or were sacrificed moribund.

Relative to the control values, dogs in Group IV consumed substantially less food during study days 3-13 (males) and 2-27 (females). These dogs were dosed with Difenzoquat as follows: 75 mg/kg/day during days 1-6; 50 mg/kg/day during days 7-8; and 44 mg/kg/day during days 9-28. After Difenzoquat was decreased from 44 to 30 mg/kg/day on study day 29, mean food consumption values were comparable to control values for the remainder of the treatment period. Difenzoquat did not affect the food consumption of male and female dogs in Groups II (12.5 mg/kg/day) and III (37.5/20 mg/kg/day).

The daily mean food consumption data for weeks 1 through 4 are in Attachment II of this review. The weekly mean food consumption data for weeks 0 through 52/54 are summarized below.

Mean Food Consumption of Male Dogs .

Group	I	II	III	IV	V
Week	Food consumption (g/kg b.w./day)				
0	37.2	49.7	43.4	46.7	44.6
1	36.8	45.5	34.4	27.5	17.0 **
2	46.7	46.6	43.3	30.3	0.2 ■
5	47.5	44.7	32.7	46.9	T
10	43.5	40.7	40.8	42.0	
20	37.9	36.8	32.8	37.1	
30	36.3	35.8	32.3	35.6	
40	35.3	34.3	31.6	33.9	
52	33.0	32.7	30.7	32.6	

This table is based on Appendix H, pages 332-338 of the submitted report (MRID No. 42800401).

** Statistically significant difference from the control group mean at the 1% level (Dunnett's t-test).

T = Terminated on day 9.

■ This value is for 2 surviving dogs which died on day 9.

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Mean Food Consumption of Female Dogs

Group	I	II	III	IV	V
Week	Food consumption (g/kg b.w./day)				
0	45.4	51.9	52.2	44.6	52.4
1	43.4	46.3	42.7	21.5 **	21.8 **
2	57.7	57.2	53.8	30.8 *	0.3 **
3	60.3	59.1	54.2	36.6	T
4	58.2	56.9	50.6	37.7	
5	58.8	55.6	53.4	53.4	
10	48.4	48.5	46.0	48.0	
20	38.9	37.0	33.3	41.4	
30	35.5	37.3	30.7	36.6	
40	33.5	28.7	31.5	38.6	
52	32.9	28.3	29.5	37.3	

This table is based on Appendix H, pages 339-345 of the submitted report (MRID No. 42800401).

* and ** Statistically significant difference from the control group mean at the 5% and 1% level, respectively (Dunnett's test).

T = Terminated on day 9.

Hematology

These data were reported as mean and individual values for each time interval when determinations were performed. Difenzoquat had no effect on the hematology parameters examined. At each time interval, the results obtained for the male and female treated groups were similar to those obtained for the respective control groups. Also, the hematology values obtained during the treatment period were similar to the pretest hematology values.

Clinical Chemistry

These data were reported as mean and individual values for each time interval when determinations were performed. Difenzoquat had no effect on the clinical chemistry parameters examined. Although a few parameters -- total bilirubin, calcium, potassium, glucose and gamma glutamyl transpeptidase (GGPT) -- were statistically significantly different from the control group means, these differences were treatment-unrelated. These data are shown below.

Test Times	Parameter	Control Value	Difference from Control
Week 6	Total bilirubin	0.3 mg/dL	Decrease to 0.1 mg/dL * in Group II (12.5 mg/kg) females.
Month 3	Calcium	10.4 mg/dL	Decrease to 9.9 mg/dL * in Group IV (30 mg/kg ■) females.
	Potassium	5.3 mEq/L	Decrease to 4.8 *, 4.6 ** and 4.6 ** mEq/L in male Groups II, III (20 mg/kg ■) and IV, respectively.
	GGPT	2.0 IU/L	Increase to 4.0 IU/L ** in Group III males.
Month 6	Fasting glucose	89 mg/dL	Increase to 102 mg/dL * in Group IV males.

This table is based on Appendix K (Mean Clinical Chemistry Values), pages 487-506 of the submitted report (MRID No. 42800401).

* and ** Statistically significant difference from the control group mean at the 5% and 1% level, respectively (Dunnett's t-test).

■ From day 29 until the termination of the study, dogs in Groups III and IV received, respectively, 20 and 30 mg of Difenzoquat / kg of body weight. The dose level for Group II (12.5 mg/kg) remained unchanged throughout the study.

Urinalysis

These data were reported as individual values for each time interval when determinations were performed. Difenzoquat had no effect on any of the urinalysis parameters examined.

Organ Weights

Organ weights were reported as (1) mean and individual absolute weights; (2) mean and individual organ/body weight ratios; and (3) mean and individual organ/brain weight ratios, for all dogs sacrificed at the termination of the study.

Difenzoquat had no effect on absolute and relative organ

weights. Although a few weights -- liver, spleen and thyroid/ parathyroid glands -- were statistically significantly different from the control group means, these defferences were incidental rather than treatment-related. These data are shown below.

Group	Organ Affected	Weight Affected	Control Value	Difference from Control
III Males	Spleen	Absolute	40 g	Increase to 70.4 g *, attributed by the testing facility to the exsanguination procedures.
		Organ/body weight ratio ■	3.51	Increase to 5.67 *
		Organ/brain weight ratio ■■	5.12	Increase to 9.43 *
III Females	Liver	Absolute	324.2 g	Decrease to 271.9 g * No significant decreases were observed in relative weights and no decreases occurred in Group IV.
IV Males	Liver	Absolute	306.6 g	Increase to 347.8 g * No increase was observed in relative weights.
	Thyroid/ Parathyroid	Absolute	0.732 g	Increase to 0.927 g * No significant increase was observed in relative weights.

This table is based on Appendix M, pages 605-610 of the submitted report (MRID No. 42800401).

* Statistically significant difference from the control group mean at the 5% level (Dunnett's t-test).

■ Spleen/body weight ratio = Spleen weight x 1000 / body weight.

■■ Spleen/brain weight ratio = Spleen weight x 10 / brain weight.

Necropsy (Macroscopic Findings)

All dogs in the study were examined. Treatment-related macroscopic findings were not observed in dogs which were sacrificed at the termination of the study and which were dosed with 12.5 (Group II), 20 (Group III) and 30 (Group IV) mg of Difenzoquat/kg of body weight from day 29. (Group II was dosed with 12.5 mg of Difenzoquat/kg of body weight from day 1).

Treatment-related macroscopic findings were observed only in the 12 dogs (5 males and 7 females) which died or were sacrificed during the first month of this study (days 1-27; there were no other deaths in this study). These dogs were dosed with 44-75 (Group IV) and 75-125 (Group V) mg of Difenzoquat/kg of body weight. The predominant findings were discoloration and/or abnormal contents in the esophagus, stomach, small intestine (duodenum, ileum, jejunum) and large intestine (cecum, colon, rectum) of some dogs. These findings were not seen in every tissue of every dog, as is shown in the table below.

Macroscopic Findings

	Group IV		Group V	
	Males	Females	Males	Females
Number of dogs examined	6	6	4	4
Finding	Number of dogs with finding			
Cecum				
Discolored	1	2	2	1
Abnormal contents	1	2	1	2
Colon				
Discolored ■	1	4	2	1
Abnormal contents	1	1	1	1
Duodenum				
Discolored	0	1	4	3
Abnormal contents	1	3	0	2
Esophagus				
Discolored	1	1	1	2
Abnormal contents	1	1	0	1
Gallbladder				
Abnormal contents	0	0	1	2

Continued on next page

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Macroscopic findings - continued

	Group IV		Group V	
	Males	Females	Males	Females
Finding	Number of dogs with finding			
Ileum				
Discolored	0	2	1	2
Abnormal contents	1	1	1	2
Jejunum				
Discolored	1	2	3	2
Abnormal contents	0	1	2	3
Lungs				
Discolored	2	4	1	3
Spleen				
Discolored	0	1	0	2
Enlarged	0	0	1	1
Stomach				
Discolored	1	4	3	3
Abnormal contents	1	2	2	3
Rectum				
Discolored	1	3	3	2
Abnormal contents	1	1	1	2

This table is based on Appendix N, TABLE II, pages 652-655 of the submitted report (MRID No. 42800401).

■ Discolored colon was also observed in 1/4 Group II male, 4/6 Group I females and 1/4 Group III females. These groups were dosed with 12.5, 0 and 37.5/20 mg of Difenzoquat/kg of body weight, respectively.

Microscopic Findings

These data were reported as (1) incidence of histopathological findings for dogs which died on the study; (2) incidence of histopathological findings for dogs sacrificed at termination of the study; and (3) very detailed individual microscopic (and macroscopic) findings for each dog on the study.

Treatment-related microscopic findings were not observed in dogs which were sacrificed at the termination of the study and

which were dosed with 12.5 (Group II), 20 (Group III) and 30 (Group IV) mg of Difenzoquat/kg of body weight.

Treatment-related microscopic lesions were observed only in male and female dogs from Groups IV and V which died on study during days 1-27 (there were no other deaths in this study). These dogs were dosed with Difenzoquat as follows: 75/50/44 mg/kg/day (Group IV) and 125/100/75 mg/kg/day (Group V). Group V was terminated on day 9, whereas Difenzoquat was reduced to 30 mg/kg/day for Group IV on day 29.

Treatment-related microscopic lesions were present in the heart and the gastrointestinal tract of both sexes. Cardiac lesions were observed in 7 of the 12 dogs that died on study and included myocardial degeneration (6 dogs) or myocardial necrosis (1 dog). A few lesions were calcified. The histomorphology of the hearts of 5 remaining dogs that died on study was within normal limits. Gastrointestinal lesions included (1) necrotic ulcer and acute inflammation of the esophagus; (2) luminal or mucoid exudate, necrotic ulcer and congestion of the stomach; and (3) congestion, exudate and necrotic ulcer of the large (cecum, colon, rectum) and small (duodenum, ileum, jejunum) intestine. The gastrointestinal lesions were not present in every tissue of every animal. The incidence of microscopic findings observed in the nonsurviving dogs is summarized below.

Microscopic Findings in Nonsurviving Dogs

	Group IV		Group V	
	Males	Females	Males	Females
Number of dogs examined	1	3	4	4
Finding	Number of dogs with finding			
Esophagus				
Acute esophagitis	0	0	1	1
Necrotic ulcer	0	0	0	2
Heart				
Calcification	1	0	1	1
Myocardial degeneration	0	2	2	2
Myocardial necrosis	0	0	0	1
Cecum				
Congestion	0	0	0	1
Lumenal exudate	0	0	0	1

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Microscopic Findings in Nonsurviving Dogs - continued

	Group IV		Group V	
	Males	Females	Males	Females
Finding	Number of dogs with finding			
Colon				
Congestion	1	1	1	2
Lumenal exudate	0	0	2	1
Rectum-Terminal Colon				
Congestion	0	1	2	2
Duodenum				
Congestion	0	1	1	1
Ileum				
Congestion	0	1	0	2
Necrotic ulcer	0	0	0	1
Jejunum				
Congestion	1	1	1	2
Lumenal exudate	0	0	1	0
Mucoid exudate	1	0	0	0
Stomach				
Congestion	1	0	1	1
Lumenal exudate	0	0	0	1
Mucoid exudate	1	0	0	0
Necrotic ulcer	0	0	1	1
Spleen				
Congestion	1	2	4	3
Kidney				
Microconcretion	1	3	4	4
Trachea				
Lumenal exudate	0	0	2	0

The above table is based on Appendix N, TABLE III, pages 658-663 of the submitted report (MRID No. 42800401).

The myocardial lesions ranged from trace to mild to moderate in severity. The gastrointestinal lesions were classified mostly as trace or mild. Microscopic findings in tissues other than heart and gastrointestinal tract were regarded by the testing

facility as physiological, spontaneous, agonal or technique-related or not unusual for dogs of this strain and age. For example, in Group IV, 1 or 2 nonsurviving dogs had (besides findings in spleen and kidney) congestion in the liver and thymus, and edyma in the mesenteric lymph node and pancreas. In Group V, 1 male and/or 1 female dog had (besides findings in spleen, kidney and trachea) lymphocytic infiltration in the salivary gland, liver and lung; and chronic interstitial pneumonia in the lung.

Microscopic findings observed at the terminal sacrifice were dose-unrelated and did not usually occur in each group. The predominant findings (2-5/group) were lymphocytic infiltration in the salivary gland, liver and lungs; lymphoid hyperplasia in the cecum; congestion in the colon and spleen; microconcretion in the kidneys; and cysts in the pituitary. Other findings were single occurrences, observed generally in the control and/or low-dose groups.

COMMENTS

This study was very well reported, but there were some problems with selection of the initial dose levels of Difenzoquat. It was not stated in the submitted report how the initial dose levels were selected. However, doses of 75/50/44 mg/kg/day (Group IV) and 125/100/75 mg/kg/day (Group V), used during the first test month, exceeded the MTD. A total of 12 dogs died or were sacrificed moribund during that time, 4 in Group IV and all (8) in Group V. In fact, Group V was terminated on day 9. The study was completed following decreases in Difenzoquat to 20 mg/kg/day (Group III) and 30 mg/kg/day (Group IV) on day 29. At Difenzoquat levels of 12.5, 20 and 30 mg/kg/day, used until the termination of the study, the only toxic signs observed were decreases in mean body weight gains of female dogs in Group IV. Starting with day 29 (week 5) until the termination of the study, these dogs did not gain as much weight as did the controls (40-64% of the control values; $p = 0.01$ or 0.05).

The following statements were included in the submission:

1. Statement of Data Confidentiality Claims.

2. Statement of Compliance with the EPA Good Laboratory Practices Regulations, 40 CFR 160, and OECD Good Laboratory Practice, ISBN 92-64-12367-9.

3. Data Flagging Statement (there are no 6a-2 data in this study).

4. Quality Assurance Statement. This study was inspected 22 times during 1/30/91 and 9/2/92. The inspections were conducted by the Quality Assurance Unit of Bio/dynamics.

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ATTACHMENT I

AVENGE (DIFENZOQUAT)

Page_____ is not included in this copy.

Pages 26 through 31 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.

X 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.

MRID No. 428004 01
Study No. 90-3640
Study Date: 5/6/93

010758

Subdivision F
Guideline Ref. No. 83-1
December 24, 1989

83-1 Chronic Feeding in the Rodent and Nonrodent (Dog)

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 20 rodents or 4 nonrodents/sex/group (3 test groups and control group).
3. ✓ Dosing duration in rodents minimum 12 month nonfood use, 24 months food use; in nonrodents minimum 12 months[1].
4. ✓ Doses tested include signs of toxicity at high dose but no lethality in nonrodents or a limit dose if nontoxic (1,000 mg/kg).
5. • ✓ Doses tested include a NOEL.
6. • ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual or cage food consumption.
10. • ✓ Opthalmoscopic examination (at least pertest and at term) control and high dose.
11. ✓ Clinical pathology data for all nonrodents and at least 10 rodents/group consisting of 12, 13 & 14.
12. ✓ Hematology at 6 month intervals consisting of at least;
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (or clotting measure)
13. ___ Clinical chemistry at 6 month intervals consisting of at least;
    - • ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - ✓ Alanine aminotransferase
    - • ✓ Creatinine kinase
    - • ✓ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - • ✓ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea nitrogen
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
14. ___ Urinalysis at 6 month intervals consisting of at least;
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ✓ Total bilirubin
    - • ✓ Urobilirubin
    - ✓ Sediment
    - ✓ Specific gravity (osmolality)
    - • ✓ Volume
15. ✓ Individual necropsy of all animals.

Criteria marked with a • are supplemental and may not be required for every study.

16. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.

✓ aorta	✓ jejunum	✓ peripheral nerve
✓ eyes	✓ bone marrow	✓ kidneys†
✓ caecum	✓ liver†	✓ esophagus
✓ colon	✓ lung	✓ ovaries
✓ duodenum	✓ lymph nodes	✓ oviduct
✓ brain†	✓ stomach	✓ pancreas
✓ skin	✓ mammary gland	✓ rectum
✓ heart	✓ spleen	✓ spinal cord (3x)
✓ testes†	✓ musculature	✓ thyroid / parathyroids
✓ pituitary	✓ epididymis	✓ salivary glands
✓ ileum	✓ adrenals	✓ thymus
✓ trachea	✓ urinary bladder	✓ accessory sex organs; uterus
✓ gall bladder		

† organs to be weighed

[1] In some cases, a six month study may be acceptable. Contact EPA to discuss the criteria for determining if such a study can be used to support reregistration.

Criteria marked with a * are supplemental and may not be required for every study.

010758

Primary Review by: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I
Health Effects Division (H7509C) Krystyna K. Locke 1/19/94

Secondary Review by: Roger Gardner, Section Head
Section I, Toxicology Branch I (H7509C)
Health Effects Division (H7509C) Roger Gardner 1/25/94

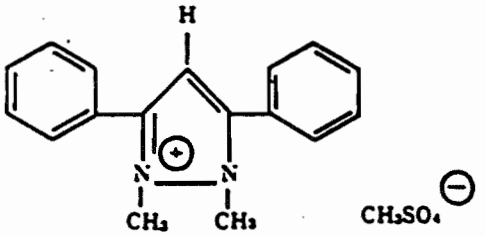
DATA EVALUATION RECORD

STUDY TYPE: 83-2b Carcinogenicity in Mice

EPA IDENTIFICATION NUMBERS:

MRID No.: 42800402	DP Barcode No.: D193941
EPA ID NO.: 106401	Submission No.: S445822
Case No.: 819395	Rereg. Case No.: 0223
P.C. Code No.: 106401	Tox. Chem. No.: 363 A

TEST MATERIAL: Technical AC 84,777 (Difenzoquat methyl sulfate; Avenge; 1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium methyl sulfate; selective herbicide); Chem. Abs. Registry No.: 43222-48-6; white crystalline powder, received at the testing facility on October 9, 1986; Lot No.: 233HA236; HLA sample No.: 61001984; purity (content of Difenzoquat); 98.7%; stable when stored in an air-tight container in the dark, dry place at room temperature (72 ± 3°F). Structure:



Difenzoquat Methyl Sulfate

REPORT NUMBER: HLA 6123-145

SPONSOR: American Cyanamid Company, Princeton, New Jersey.

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, Wisconsin.

TITLE OF REPORT: Chronic Dietary Toxicity and Oncogenicity Study with AC 84,777 in Mice.

AUTHOR: Karen M. MacKenzie

STUDY COMPLETION DATE: June 23, 1989

010758

SUMMARY:

Groups of Crl:CD-1(ICR)BR mice (55/sex/dose) were administered Difenzoquat for 79/80 weeks at dietary levels of 0, 200, 500 and 1000 ppm (0, 26.9, 69.4 and 150.1 mg/kg/day* for males and 0, 39.7, 97.9 and 202.4 mg/kg/day* for females). In addition, 10 mice/sex/dose, receiving the same diets, were sacrificed during weeks 53-54. * Mean achieved doses.

Almost 59-65% of male mice and 59-66% of female mice survived to scheduled termination of the study. The only treatment-related and statistically significant (p=0.05) toxic sign observed was a decrease in body weight gain in the high-dose males (62% maximum) and females (54% maximum), and in the mid-dose males (34% maximum). Difenzoquat was not carcinogenic in this study. The number of tumor-bearing male and female mice was lower in the treated groups than in the controls. Pulmonary adenoma was the most common neoplasm.

Systemic NOEL: 200 ppm (26.9 mg/kg/day); males
500 ppm (97.9 mg/kg/day); females

Systemic LOEL: 500 ppm (69.4 mg/kg/day); males [decreased body weight gains]

1000 ppm (202.4 mg/kg/day); females [decreased body weight gain]

Classification: Core-Guideline

EXPERIMENTAL PROCEDURES

The mice (weanlings) were received in the testing facility on March 9, 1987; dosing was initiated on March 31, 1987; interim sacrifice took place on April 4-5, 1988; and terminal sacrifice occurred during September 30-October 11, 1988.

Crl:CD-1(ICR)BR mice received AC 84,777 in the diet for 79/80 weeks. There were initially 65 mice/sex/group, but 10 mice/sex/group were sacrificed after one year of treatment (interim sacrifice during week 53). The dose levels used, corrected for purity of technical Difenzoquat, were 0, 200, 500 and 1000 ppm (Groups 1, 2, 3 and 4, respectively). It was not reported on what basis these doses were selected. The diets were prepared weekly during the course of the study; each dose level was prepared independently. AC 84,777 was added to the commercial diet (Certified Purina Rodent Chow #5002; Purina Mills, Inc.) on the weight-to-weight basis. To ensure adequate mixing, AC 84,777 was first mixed with small amounts of the commercial diet to prepare a premix. The resulting premix was then thoroughly blended with more of the commercial diet to obtain the appropriate concentrations of AC 84,777. Mixed diets were stored refrigerated in closed polyethylene containers. The test diets were not pelleted.

According to the author of this study, Crl:CD-1(ICR)BR strain of mice was used because this strain was sensitive to chmical oncogens of various types. Also, the testing facility had historical data on the tumor incidence and survival from mice of this strain and source. The mice were:

(1) Obtained from the Portage, Michigan, facility of Charles River Laboratories (Wilmington, Massachusetts).

(2) Acclimated for 22 days before random assignment to groups.

(3) Housed individually in stainless steel, screen-bottom cages at temperatures of 72 ± 3°F, relative humidity of 50 ± 20% and 12 hours light/12 hours dark cycles. Air changes/hour were not reported.

(4) Identified by number tattoo and by cage and group number.

(5) Allowed free access to food and water (via an automatic system).

Individual body weights at the time of randomization did not exceed ± 2 standard deviations from the mean body weight for each

sex, and group mean body weights for each sex were not statistically different at the 5% level.

The following parameters were examined for all mice on the study unless indicated otherwise:

(1) **Clinical Observations:** Twice daily (a.m. and p.m.) for signs of toxicity (e.g. changes in appearance, behavior, gait and excretory functions), moribundity, or death. Once a week, each animal was removed from its cage and examined for abnormalities and clinical signs of toxic effects.

(2) **Body Weights:** Before initiation of treatment, on the first day of treatment, weekly through week 14, once every 2 weeks from week 16 through week 26, monthly thereafter, and on the day of sacrifice.

(3) **Food Consumption:** Weekly through week 14, once every 2 weeks from week 16 through week 26, and monthly thereafter to the end of the study.

(4) **Hematology:** During weeks 53 and 79, using 10 animals (fasted overnight)/sex/group. Blood was collected from the retro-orbital venous plexus. The animals selected for the week 53 bleeding were the same animals that were selected for the interim sacrifice during week 53. The following tests were done:

White blood cell and differential white cell count
Red blood cell and platelet count
Hemoglobin and hematocrit

The following values were calculated:

Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Mean corpuscular volume

(5) **Necropsy:** All mice on the study were necropsied, including the nonsurvivors (mice sacrificed in a moribund condition) and those found dead during the course of the study. The necropsy included a macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the external surfaces of the brain and spinal cord; the nasal cavity and paranasal sinuses; and the thoracic, abdominal and pelvic cavities, and viscera.

(6) Organ Weights (Absolute and Relative): At the scheduled sacrifices (interim and terminal), the following organs were weighed from 10 mice/sex/group: adrenals, brain, gonads, heart, kidneys, liver, lungs, pituitary, spleen, and thyroids with parathyroids. Relative weights (organ-to-body weight percentages and organ-to-brain weight ratios) were also calculated. At the termination of the study, organs to be weighed were obobtained from the 10 mice/sex/group which were used for the hematological determinations.

(7) Histopathology: The following tissues were examined for all mice on the study:

Adrenals
Aorta
Bladder, urinary
Bone with marrow (sternal)
Brain
Esophagus
Eyes
Gallbladder
Heart
Intestine, large (cecum and colon)
Intestine, small (duodenum, jejunum and ileum)
Kidneys
Liver
Lungs (with mainstem bronchi)
Lymph nodes (mediastinal and mesenteric)
Mammary gland
Muscle, skeletal
Any other tissue with gross lesions
Nerve, sciatic
Optic nerve
Ovaries
Pancreas
Pituitary
Prostate
Salivary gland
Seminal vesicles
Skin (mammary area)
Spinal cord (lumbar and thoracic)
Spleen
Stomach
Testes
Thymus
Thyroid with parathyroid
Tongue
Trachea
Uterus (corpus and cervix)
Vagina

The above tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. Before examination, eyes (obtained only at scheduled sacrifices) were preserved in Zenker's solution and other tissues were preserved in 10% phosphate-buffered formalin.

Other parameters examined in this study were:

(1) Concentration of Difenzoquat in the Diets: A sample of approximately 100 g of each diet was frozen and sent to the Sponsor for analysis. For the first 8 weeks of the study, all diet samples were assayed. Thereafter,

a random-number table generated by a BASIC program called RANDOM on the VAX computer was used to select one dose level per week. Duplicate 20-g diet samples were assayed by the American Cyanamid Company HPLC Method M1693. This method is validated for AC 84,777 in rodent meal from 200 to 1000 ppm. The sensitivity of this method is 50 ppm.

(2) Stability of Difenzoquat in Powdered Diets Stored at Room Temperature (72 ± 3° F) or Refrigerated: These determinations were performed for one diet batch, for the low-dose (200ppm) and high-dose (1000 ppm) groups, over the 14-day period, just before study initiation. The diets were prepared on March 23, 1987 and analyzed immediately. The analyses were then repeated 7 and 14 days later. Diet samples stored at room temperature were in mouse feeders, in the animal room. Samples stored refrigerated were in lidded containers. The preparation, storage and sampling of diets were done by the testing facility, but the analyses were performed by the Sponsor where samples (frozen) were sent.

(3) Homogeneity of Diet Mixes with Respect to Difenzoquat: These determinations were performed on diets prepared for the low-dose and high-dose groups on March 23, 1987 and used for stability studies. Diet samples were taken from the right and left side of the top, middle and bottom of Hobart blender bowl and analyzed for the content of Difenfzoquat. The analyses were performed by the Sponsor.

Statistical Analyses:

Body weights, cumulative body weight gains, food consumption, hematological data, organ weights, organ-to-body weight percentages and organ-to-brain weight ratios were analyzed statistically as described in Attachment I of this review. Histopathological data were not analyzed statistically because, according to the submitted report, none of the tumors cited met the criteria for performing statistical analyses.

RESULTS

Achieved Concentrations of Difenzoquat in Diets

Twenty three batches of control diets and 78 batches of Difenzoquat-containing diets (26 per dose) were analyzed during weeks 1 through 80. Difenzoquat was not detected in any of the control diets. The overall mean concentrations of Difenzoquat in the diets of the treated groups were within 0.2-4.5% of nominal

values. These data are summarized below.

Nominal dietary concentrations of Difenzoquat (ppm)	200	500	1000
Mean achieved concentrations of Difenzoquat (ppm)	191	499	992
Standard deviation	11.0	46.7	53.3
95% Confidence limits	137-196	480-528	971-1014
Percent of nominal concentrations of Difenzoquat achieved	95.5	99.8	99.2
Mean doses of Difenzoquat achieved (mg/kg/day): **males**	**26.9**	**69.4**	**150.1**
Standard deviation	4.58	12.95	27.45
Range	21.1 to 38.7	54.0 to 103.7	119.3 to 220.0
Mean doses of Difenzoquat achieved (mg/kg/day): **females**	**39.7**	**97.9**	**202.4**
Standard deviation	7.87	22.98	45.28
Range	26.6 to 54.6	63.9 to 133.5	137.4 to 290.1

This table is based on data reported on page 25 and on TABLE I, page 1357, of the submitted report (MRID No. 42800402).

According to the above table, the consumption of Difenzoquat (mg/kg/day) by females was 47.6, 41.1 and 34.8%, respectively, higher than by the males.

Stability of Difenzoquat in Diets

Difenzoquat was stable in diets stored in animal feeders, in the animal room, and in diets stored refrigerated, in the closed containers. The duration of storage in each instance was 14 days (diets were prepared weekly during the course of the study). On storage day 14 at room temperature, the mean concentrations of Difenzoquat in the 200 ppm and 1000 ppm diets were 94% and 95%, respectively, of the nominal concentrations. For diets stored refrigerated, the mean concentrations of Difenzoquat in the 200 ppm and 1000 ppm diets were 96% and 97%, respectively, of the nominal concentrations. The mid-dose diet (500 ppm) was not

analyzed for stability.

Homogeneity of Difenzoquat in Diets

The homogeneity of Difenzoquat in the low-dose and high-dose diets was satisfactory. The mean concentrations of Difenzoquat in the top, middle and bottom of the low-dose diet batch were 94.0, 94.9 and 92.7%, respectively, of the theoretical concentration (200 ppm). The mean concentrations of Difenzoquat in the top, middle and bottom of the high-dose diet batch were 95.5, 92.8 and 93.1%, respectively, of the theoretical concentration (1000 ppm). The mid-dose diet (500 ppm) was not analyzed for homogeneity of Difenzoquat.

Clinical Observations

As in any study of this type, a variety of findings were observed in all groups, including the controls, but all were dose-unrelated. The most frequent findings were rough hair coats, alopecia, urine stains, bloody crusts, red ears, small movable tissue masses (only in males) and convulsions. Most mice with convulsions lived to the terminal sacrifice. It was reported when (test week) convulsions were first observed in each animal, but the severity and duration of convulsions were not reported. The incidence and time of occurrence of convulsions, and the fate of mice with convulsions, are summarized below.

Difenzoquat (ppm)	0	200	500	1000
Number of mice with convulsions:				
Males	24	19	20	21
Females	24	28	18*	20
Percent of mice with convulsions: **				
Males	37	29	31	32
Females	37	43	28	31
Percent of mice with convulsions noted during the first year (weeks 9-52):				
Males	67	58	60	62
Females	29	46	67	35

Continued on next page.

Difenzoquat (ppm)	0	200	500	1000
Percent of mice with convulsions noted during weeks 53-78:				
Males	33	42	40	38
Females	71	54	33	65
Percent of mice with convulsions living to terminal sacrifice:				
Males	83	68	70	67
Females	67	68	78	85
Percent of mice with convulsions sacrificed moribund, accidentally, after 1 year (interim sacrifice), or found dead: Males	17	32	30	33
Females	33	32	22	15
Moribund sacrifice (%)				
Males	8	5	10	0
Females	8	18	5	10
Accidental death (%)				
Males	0	0	0	5
Interim sacrifice (%)				
Males	4	16	15	5
Females	8	7	11	0
Found dead (%)				
Males	4	11	5	24
Females	17	7	5	5

This table is based on APPENDIX B (Individual Antemortem Observations), pages 286-347, of the submitted report (MRID No. 428004-02).

* According to Table 1 (Summary of Antemortem Observations) of the submitted report, page 37, there were 19 mice with convulsions in this group. However, only 18 could be counted in APPENDIX B.

** There were 65 male and 65 female mice in each group, and these numbers were used by the toxicologist who reviewed this study (KKL) to calculate percentages.

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Mortality

Difenzoquat had no effect on mortality. Almost 59% to 65% of male mice and 59% to 66% of female mice survived to scheduled termination of the study. In general, mortality was low in all four groups through week 62 but increased thereafter, especially in male groups 1 and 2, and in female groups 1, 2 and 3. At the termination of the study, survival was similar in the control groups, male and female, and the Difenzoquat-treated groups. The mortality data are summarized below.

Difenzoquat (ppm)	0	200	500	1000
	Number of mice killed or found dead			
Terminal sacrifice				
Males	39	38	42	40
Females	42	39	38	43
Interim sacrifice				
Males	10	10	10	10
Females	10	10	10	10
Unscheduled deaths				
Males	16	17	13	15
Females	13	16	17	12
Found dead				
Males	8	12	6	12
Females	8	6	14	6
Moribund sacrifice				
Males	7	4	7	2
Females	5	10	2	6
Accidental deaths				
Males	1	1	0	1
Females	0	0	1	0
	Percent of mice killed or found dead *			
Terminal sacrifice				
Males	60.0	58.5	64.6	61.5
Females	64.6	60.0	58.5	66.1
Interim sacrifice				
Males	15.4	15.4	15.4	15.4
Females	15.4	15.4	15.4	15.4
Unscheduled deaths				
Males	24.6	26.1	20.0	23.1
Females	20.0	24.6	26.1	18.5

This table is based on Table 1 (Summary of Antemortem Observations), page 40, and on APPENDIX D (Individual Animal Pathology Data), pages 549-1332, of the submitted report (MRID No. 428004 02). * Based on 65 mice/sex/group.

The interim sacrifice took place during weeks 53 and 54, whereas the terminal sacrifice occurred during weeks 79-81. The number of mice sacrificed at various times is shown below.

Difenzoquat (ppm)	0	200	500	1000	Total killed
Interim sacrifice					
Week 53 - Males	7	3	5	6	21
Females	5	3	6	5	19
Week 54 - Males	3	7	5	4	19
Females	5	7	4	5	21
Terminal sacrifice					
Week 79 - Males	10	10	10	10	40
Females	10	10	10	10	40
Week 80 - Males	25	25	28	24	102
Females	25	23	25	27	100
Week 81 - Males	4	3	4	6	17
Females	7	6	3	6	22
Total - Males	39	38	42	40	159
Females	42	39	38	43	162

This table is based on APPENDIX D (Individual Animal Pathology Data), pages 549-1332, of the submitted report (MRID No. 428004-02).

Mice which were sacrificed in extremis or were found dead, both controls and Difenzoquat-treated, had two or more of the following: low body temperature, swollen body, hunched posture, exophthalmus, small movable tissue masses (males only), tremors, convulsions and head tilt, and were thin and cyanotic. However, convulsions were also observed frequently in the mice which lived to study termination. Other toxic signs, observed in the nonsurviving mice, were also observed in the survivors but with lesser frequency. Data summarized below indicate when unscheduled deaths occurred during the study.

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Study weeks		1-25	26-52	53-62	63-72	73-80	
Difenzoquat (ppm)		Number of mice sacrificed in extremis and found dead					Total
0:	Males	0	2	3	3	8	16
	Females	1	0	2	4	6	13
200	Males	0	3	4	8	2	17
	Females	0	2	1	5	8	16
500	Males	2	5	1	2	3	13
	Females	1	0	1	10	5	17
1000	Males	0	2	4	4	5	15
	Females	2	1	3	3	2	12

This table is based on APPENDIX D (Individual Pathology Data), pages 549-1332, of the submitted report (MRID No. 42800402).

Body Weights

These data were reported as weekly mean body weights and body weight gains, and as weekly individual body weights for weeks 1 through 78. The terminal body weights (weeks 79, 80 or 81) were reported in the individual animal pathology data.

Compared with the control values, there was a statistically significant reduction in mean body weights of the high-dose (1000 ppm) males throughout the study, and of the high-dose females during weeks 4, 9, 30, 34, 46, 58-62 and 70-78. The maximum reduction in body weight of males was 16% and occurred during week 74. The maximum reduction in body weight of females was 10% and occurred during week 74. Difenzoquat had no effect on body weights of the mid-dose and low-dose males and females.

Relative to the control values, the mean percent decreases in body weight gains of the low-dose, mid-dose and high-dose males were 0-16, 2-34 and 30-62, respectively. The corresponding weight gain decreases for the females were 0-27, 0-12 and 0-54%, respectively. Of the 33 body weight gain determinations reported, 1, 16 and 33 for the low-dose, mid-dose and high-dose males, respectively, were statistically significantly lower than those for the controls. The corresponding values for the females were 2, 0 and 19, respectively. Based on these data, Difenzoquat affected body weight gains of the high-dose males and females, had some effect on body weight gains of the mid-dose males, and no effect on body weight gains of the mid-dose females and low-dose males and females. The body weight data are summarized below.

Mean Body Weights and Body Weight Gains of Male Mice

Difenzoquat (ppm)	0	200	500	1000
Week	Body weights (g)			
2	29.7	29.9	29.9	27.7 *
3	30.8	30.6	30.1	28.2 *
5	31.3	30.8	31.7	29.2 *
8	34.1	32.9 *	33.3	31.3 *
14	36.3	35.5	35.3	33.1 *
20	37.3	36.4	36.2	33.6 *
30	38.7	37.4	37.9	34.4 *
42	39.6	38.5	38.9	34.9 *
54	40.9	39.3	39.6	35.8 *
66	40.6	40.2	39.7	35.7 *
74	41.3	39.5	39.1	34.6 *
78	40.2	39.1	38.9	35.0 *
Week	Percent body weight decreases relative to control values #			
2	-	0	0	7
3	-	1	2	8
5	-	2	0	7
8	-	4	2	8
14	-	2	3	9
20	-	2	3	10
30	-	3	2	11
42	-	3	2	12
54	-	4	3	12
66	-	1	2	12
74	-	4	5	16
78	-	3	3	13
Week	Body weight gains (g)			
2	2.4	2.7	2.1	0.9 *
3	3.5	3.4	2.3 *	1.4 *
5	4.0	3.6	3.9	2.4 *
8	6.8	5.7 *	5.5 *	4.5 *
14	9.0	8.4	7.5 *	6.3 *
20	10.0	9.3	8.4 *	6.8 *
30	11.4	10.3	10.0	7.6 *
42	12.3	11.3	11.2	8.2 *
54	13.5	12.2	11.8	8.9 *
66	13.1	13.1	11.9	8.9 *
74	13.6	12.6	11.3 *	7.8 *
78	12.7	12.2	11.1	8.1 *

Mean Body Weights and Body Weight Gains of Male Mice - continued

Difenzoquat (ppm)	0	200	500	1000
Week	Percent body weight gain decreases relative to control values #			
2	-	0	12	62
3	-	3	34	60
5	-	10	2	40
8	-	16	19	34
14	-	7	17	30
20	-	7	16	32
30	-	10	12	33
42	-	8	9	33
54	-	10	13	34
66	-	0	9	32
74	-	7	17	43
78	-	4	13	36

This table is based on Table 2 (Summary of Body Weight Data - Males), pages 41-44, and Table 4 (Summary of Cumulative Body Weight Gain Data - Males), pages 49-52, of the submitted report (MRID No. 42800402).

Calculated by the testing facility.

* Statistically significant difference from the control group mean at the 5% level (Dunnett's t-test).

Mean Body Weights and Body Weight Gains of Female Mice

Difenzoquat (ppm)	0	200	500	1000
Week	Body weights (g)			
2	23.9	23.8	23.7	23.4
4	24.5	24.2	24.2	23.2 *
9	27.5	27.0	27.3	26.5 *
14	28.5	28.6	28.8	28.5
16	29.6	29.1	29.3	28.8
20	29.7	29.7	29.6	29.0
30	31.5	31.1	31.4	30.2 *
34	31.9	31.7	31.8	30.2 *
46	33.2	33.2	32.7	31.3 *
58	34.2	33.9	33.7	31.7 *
62	34.4	34.4	34.1	32.4 *
70	34.5	34.4	33.4	31.7 *
74	34.7	34.7	33.7	31.2 *
78	34.1	34.8	33.8	31.3 *
Week	Percent body weight decreases relative to control values #			
2	-	0	1	2
4	-	1	1	5
9	-	2	1	4
14	-	0	0	0
16	-	2	1	3
20	-	0	0	2
30	-	1	0	4
34	-	1	0	5
46	-	0	2	6
58	-	1	1	7
62	-	0	1	6
70	-	0	3	8
74	-	0	3	10
78	-	0	1	8
Week	Body weight gains (g)			
2	1.9	1.5	1.9	1.4 *
4	2.6	1.9	2.3	1.2 *
9	5.6	4.8 *	5.4	4.6 *
14	6.5	6.4	6.9	6.5
16	7.7	6.9 *	7.4	6.9 *
20	7.8	7.4	7.8	7.1
30	9.5	8.9	9.5	8.3 *
34	10.0	9.4	9.9	8.3 *
46	11.3	10.9	10.9	9.4 *

Mean Body Weights and Body Weight Gains of Female Mice - continued 010758

Difenzoquat (ppm)	0	200	500	1000
Week	Body weight gains (g) - continued			
58	12.3	11.8	11.8	9.9 *
62	12.5	12.3	12.3	10.6 *
70	12.6	12.2	11.5	9.9 *
74	12.8	12.5	11.8	9.6 *
78	12.2	12.7	11.9	9.6 *
Week	Percent body weight gain decreases relative to control values #			
2	-	21	0	26
4	-	27	12	54
9	-	14	4	18
14	-	2	0	0
16	-	10	4	10
20	-	5	0	9
30	-	6	0	13
34	-	6	1	17
46	-	4	4	17
58	-	4	4	20
62	-	2	2	15
70	-	3	9	21
74	-	2	8	25
78	-	0	2	21

This table is based on Table 3 (Summary of Body Weight Data - Females), pages 45-48, and Table 5 (Summary of Cumulative Body Weight Gain Data - Females), pages 53-56, of the submitted report (MRID No. 42800402).

Calculated by the testing facility.

* Statistically significant difference from the control group mean at the 5% level (Dunnett's t-test).

Food Consumption

These data were reported as weekly mean food consumption (g/mouse) and as weekly individual food consumption (g/mouse) for weeks 1 through 78. A total of 33 determinations of food consumption were reported for each sex.

Compared with the control values, Difenzoquat had no effect on food consumption of any of the treated groups. In most instances, the Difenzoquat-treated mice, especially the females, consumed more food than did the control female mice. Also, the female mice generally consumed more food than did the male mice. Only on four occasions was the food consumption of the male mice statistically significantly lower ($p = 0.05$) than that of the control male mice, as follows: in the low-dose group, during week 38 (8%) and in the high-dose group, during weeks 38, 50 and 54 (8, 6 and 9%, respectively). The food consumption data are summarized below.

Mean Food Consumption (g/mouse) During the Time Intervals Shown

Difenzoquat (ppm)	0	200	500	1000
Week 1 - 10				
Males	33.8-40.0	34.2-39.1	34.7-41.9	34.6-42.5
Females	40.9-43.0	41.4-46.8	43.1-47.4	42.3-47.2
Week 11 - 26				
Males	31.3-34.4	30.8-34.2	31.8-35.4	31.5-36.9
Females	36.5-41.9	39.0-42.7	38.7-43.0	38.4-45.2
Week 27 - 62				
Males	30.4-34.1	29.5-33.5	30.9-33.6	30.6-33.2
Females	31.5-36.6	34.3-40.7	31.6-38.5	31.4-44.3
Week 63 - 78				
Males	31.5-32.6	29.3-30.9	30.1-32.3	29.8-31.9
Females	30.5-31.6	32.2-35.3	30.1-32.3	31.0-32.3

This table is based on Table 6 (Summary of Food Consumption Data - Males), pages 57-60, and Table 7 (Summary of Food Consumption Data - Females), pages 61-64, of the submitted report (MRID No. 42800402).

Food utilization was not reported.

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Hematology

Difenzoquat had no effect on the hematological parameters examined. Although a few parmeters -- red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) -- were statistically significantly different from the control group means, these differences were both treatment-unrelated and within the normal biological variations. # These data are shown below.

Test Week	Parameter	Control Value	Difference From Control
53	MCV	50 FL	Increase to 52 FL * (4%) in the 200 ppm female group.
79	MCV	50 FL	Decrease to 48 FL * (4%) in the 1000 ppm male group.
79	RBC	9.47 E6/UL	Decrease to 7.54 E6/UL * (20%) in the 500 ppm female group.
79	HCT	47.3%	Decrease to 35.8% * (11.5 %) in the 500 ppm female group.
79	MCHC	30.2%	Increase to 31.6% * (1.4 %) in the 500 ppm female group.

This table is based on Tables 10-13, pages 67-74, of the submitted report (MRID No. 42800402).

Representative Historical Control Data (1983); Hazleton Laboratories America, Inc., Vienna, Virginia; Part XI: Hematology Reference Ranges.

* Statistically significant difference from the control group mean at the 5% level (Dunnett's t-test).

Organ Weights

Organ weights were reported as mean absolute weights (g) and as mean relative weights (organ-to-body weight percentages and organ-to-brain weight ratios) for the test weeks 53 (interim sacrifice) and 79/80 (terminal sacrifice). At each time interval, organs were weighed from 10 mice/sex/group.

Difenzoquat had no effect on organ weights. Although the high-dose males, sacrificed during week 53, had statistically significant decreases in the mean absolute and relative weights of the spleen, this was not observed at the terminal sacrifice (that is, after longer exposure to Difenzoquat). At the terminal sacrifice, the relative weights of brain and heart were statistically significantly increased in the high-dose males, but this was attributed by the testing facility to decreases in body weights. In the females, statistically significant increases were observed in the relative weights of brain, heart and thyroids, at the terminal sacrifice, but only in the mid-dose group. These increases were also attributed by the testing facility to decreases in body weights. The statistically significant differences, from the control group means, in organ weights are summarized below.

Difen-zoquat	Test Week	Sex	Organ Affected	Control Value	Difference From Control
			Absolute organ weights		
500 ppm	53	F	Heart	0.1843 g	Incr. to 0.2194 g *
1000 ppm	80	M	Spleen	0.2074 g	Decr. to 0.0610 g *
			Organ-to-body weight percentages		
500 ppm	80	F	Brain	1.8035	Incr. to 2.0562 *
500 ppm	80	F	Heart	0.6198	Incr. to 0.8364 *
500 ppm	80	F	Thyroid	0.0117	Incr. to 0.0269 *
1000 ppm	53	M	Spleen	0.7198	Decr. to 0.1974 *
1000 ppm	80	M	Brain	1.5159	Incr. to 1.7510 *
1000 ppm	80	M	Heart	0.6108	Incr. to 0.7283 *
			Organ-to-brain weight ratios		
500 ppm	80	F	Heart	0.3431	Incr. to 0.4114 *
500 ppm	80	F	Thyroid	0.0064	Incr. to 0.0134 *
1000 ppm	53	M	Spleen	0.4029	Decr. to 0.1177 *

This table is based on Tables 14-19, pages 75-92, of the submitted report (MRID No. 42800402).

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* Statistically significant difference from the control group mean at the 5% level (Dunnett's t-test).

" Thyroid " should actually read " Right thyroid/parathyroid ".

" Test week 80 " should actually read " Test week 79/80 ".

Incr. = Increase Decr. = Decrease

Necropsy (Macroscopic Findings)

All mice in the study were examined and treatment-related findings were not observed. Predominant but dose-unrelated findings were observed in the mesenteric lymph node, ovaries and uterus, whereas less predominant findings were noted in the kidneys, spleen and stomach. In most instances, the lowest incidence of findings was observed in the high-dose group. The incidence (number of mice affected per group) of these findings is summarized below.

Finding	Males		Females	
	Control	Treated	Control	Treated
Interim Sacrifice #				
Uterus: Cysts	-	-	2	2-6
Thickened walls	-	-	6	4-7
Ovaries: Cysts	-	-	5	3-8
Terminal Sacrifice ##				
Kidneys: Cysts	3	2-9	2	0-3
Rough surface	4	1-3	2	0-4
Stomach: Thickened wall	3	1-3	2	3-7
Mesenteric lymph node: Mottled	8	5-8	14	4-8
Uterus: Cysts	-	-	22	20-24
Thickened walls	-	-	15	11-15
Large, diffuse	-	-	5	4-5
Ovaries: Cysts	-	-	22	21-26
Unscheduled Deaths ###				
Kidneys: Rough surface	1	1-2	2	3-4
Large pelvis	3	1-2	2	1-3
Spleen: Enlarged	3	3-5	3	0-4
Uterus: Cysts	-	-	3	3-5
Thickened walls	-	-	5	2-5
Masses	-	-	3	1-5
Ovaries: Cysts	-	-	5	2-10

This table is based on Tables 20-22, pages 93-143, of the submitted report (MRID No. 42800402).

The numbers of mice examined macroscopically in the 0, 200, 500 and 1000 ppm groups were:

\# 10/sex/group
Males: 39, 38, 42 and 40
Females: 42, 39, 38 and 43
Males; 16, 17, 13 and 15
Females: 13, 16, 17 and 12

Microscopic Findings - Non-neoplastic

Difenzoquat did not appear to induce any non-neoplastic findings in this study, but might have enhanced amyloidosis in the high-dose (1000 ppm) male group. The most common non-neoplastic changes were (1) amyloidosis in various organs of males and females; (2) thymic involution and inflammatory changes in the kidneys and livers of both sexes; (3) subcapsular cell hyperplasia in the adrenals, mostly in the females; (4) cystic endometrial hyperplasia of the uterus; and (5) ovarian cysts. The less common non-neoplastic findings, observed in both sexes, were (a) mineralization in the brain; (b) lens missing from one eye; (c) follicular cyst(s) in the thyroid; (d) erosion/ulceration and hyperplasia of glandular mucosa in the stomach; (e) mononuclear cell infiltration in the salivary glands; (f) lymphoreticular hyperplasia in the mandibular/cervical lymph node and in the thymus (males only); and (g) congestion and pigment in the mesenteric lymph node.

The above findings were observed at the interim, terminal and unscheduled sacrifices and, with the exception of the amyloidosis in the high-dose males, were dose-unrelated. The incidence of amyloidosis in the 1000 ppm male mice was higher than that in the male control group or in the other two Difenzoquat-treated male groups. The testing facility considered this finding to be fortuitous. However, since the increased incidence of amyloidosis was observed in almost every organ examined, Difenzoquat, at the 1000 ppm level, might have enhanced amyloidosis in the male mice. In the high-dose females, the incidence of amyloidosis was generally lower than that in the other groups, including the control group. Amyloidosis in various strains of mice is accociated with aging. ■ The incidence of the most common non-neoplastic findings, observed in this study, is summarized in Attachment II.

■ Burek, J.D., Molello, J.A. and Warner, S.D. (1982). Selected Nonneoplastic Diseases. *In* " The Mouse in Biomedical Research" (H.E. Foster, J.D. Small and J.G. Fox, eds.), pp. 425-440. Academic Press, New York.

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Microscopic Findings - Neoplastic

Difenzoquat was not carcinogenic in this study. The number of the tumor-bearing male mice was lower in the treated groups than in the control group. In the females, there was a slight increase in the number of the tumor-bearing mice in the low-dose group and a decrease in the mid-dose and high-dose groups, relative to the control group. Most of the male tumor-bearing mice had single, benign tumors and only 2 mice (1 control and 1 low-dose) had multiple tumors (3 or more per animal). Also, the percentage of the tumor-bearing male mice was about the same at the scheduled and unscheduled (moribund) sacrifices. In the female groups, about two-thirds of the tumor-bearing control mice, about one-half of the tumor-bearing low-dose and mid-dose mice, and about one-third of the tumor-bearing high-dose mice had single, benign tumors. Also, 19 female mice (3-7 per group) had multiple tumors and the percentage of the tumor-bearing female mice was higher at the unscheduled than scheduled sacrifices. The percentage of mice with 2 tumors per animal was about the same in all male and female groups, including controls, and ranged from 0 to 3.1% per group. There were only 2 tumor-bearing mice (both in the low-dose male group) at the interim sacrifice. The overall incidence of tumors and the type and incidence of the primary benign and malignant tumors are shown below. The type and incidence of the metastatic, locally invasive and other tumors are in Attachment III of this review.

Distribution of Tumor-Bearing Mice Among Groups

Difenzoquat (ppm)	0	200	500	1000
	Numerical incidence			
Tumor-bearing mice				
Males	16	12	7	5
Females	13	17	12	10
Mice with single tumors				
Males	13	11	5	4
Females	8	9	6	3
Mice with two tumors				
Males	2	0	2	1
Females	2	1	2	2
Mice with multiple tumors				
Males #	1	1	0	0
Females ##	3	7	4	5

Continued on next page

Distribution of Tumor-Bearing Mice Among Groups - continued

Difenzoquat (ppm)	0	200	500	1000
	Numerical incidence			
Mice with benign tumors				
Males	13	7	5	4
Females	9	9	6	3
Mice with malignant tumors				
Males	3	3	2	1
Females	4	11	6	6
	Percent incidence ■			
Tumor-bearing mice				
Males	24.6	18.5	10.8	7.8
Females	20.0	26.2	18.5	15.4
Mice with single tumors				
Males	20.0	16.9	7.8	6.2
Females	12.3	13.9	9.2	4.6
Mice with two tumors				
Males	3.1	0.0	3.1	1.5
Females	3.1	1.5	3.1	3.1
Mice with multiple tumors				
Males #	1.5	1.5	0.0	0.0
Females ##	4.6	10.8	6.2	7.7
Mice with benign tumors				
Males	20.0	10.8	7.7	6.2
Females	13.9	13.9	9.2	4.6
Mice with malignant tumors				
Males	4.6	4.6	3.1	1.5
Females	6.2	16.9	9.2	9.2

This table is based on Table 30, pages 257-265, of the submitted report (MRID No. 42800402). Number of mice examined in each group: 65 males and 65 females.

■ Calculated by the toxicologist (KKL) who evaluated this study.

3-4 tumors per mouse.

3-26 tumors per mouse.

Distribution of Tumors-Bearing Mice at Scheduled and Unscheduled Sacrifices

Difenzoquat (ppm)	0	200	500	1000
Interim Sacrifice				
Males - No. sacrificed	10	10	10	10
No. with tumors	0	2	0	0
% with tumors	0	20	0	0
Females - No. sacrificed	10	10	10	10
No. with tumors	0	0	0	0
% with tumors	0	0	0	0
Terminal Sacrifice				
Males - No. sacrificed	39	38	42	40
No. with tumors	11	7	6	4
% with tumors	28	18	14	10
Females - No. sacrificed	42	39	38	43
No. with tumors	9	9	7	6
% with tumors	21	23	18	14
Unscheduled Sacrifice				
Males - No. sacrificed	16	17	13	15
No. with tumors	5	3	1	1
% with tumors	31	18	8	7
Females - No. sacrificed	13	16	17	12
No. with tumors	4	8	5	4
% with tumors	31	50	29	33

This table is based on Table 24 (page 158), Table 26 (page 187), Table 28 (page 217) and Table 30 (pages 257-265) of the submitted report (MRID No. 42800402).

Overall Incidence of Tumors (Neoplasms)

Difenzoquat (ppm)	0	200	500	1000
Neoplasm	Number of neoplasms observed in a group			
Primary benign				
Males	14	7	6	5
Females	11	9	7	3
Primary malignant				
Males	3	3	2	1
Females	4	11	6	7
Metastatic [redacted]				
Males	1	0	1	0
Females	19	8	1	7
Locally invasive [redacted]				
Males	2	0	0	0
Females	0	4	0	7
Other [redacted]				
Males	0	3	0	0
Females	25	42	42	14

This table is based on Table 30, pages 257-265, of the submitted report (MRID No. 42800402).

[redacted] Malignant fibrous histiocytoma in the males and carcinoma, hemangiosarcoma, sarcoma and thymic lymphoma in the females. In the control group, the 19 tumors were observed in two females, 11 in one sacrificed moribund during week 70 and 8 in another found dead during week 76.

[redacted] Mengiosarcoma in the males and thymic lymphoma in the females.

[redacted] Malignant lymphoma was the only tumor in the category of "other neoplasms". In the control group, the 25 tumors were observed in one female, sacrificed moribund during week 76. In the low-dose females, most tumors (38) occurred in three mice which were sacrificed moribund or found dead during weeks 75-80. In the mid-dose females, 28 tumors were noted in two mice which died during weeks 74-78 and 14 tumors were noted in two mice at the terminal sacrifice. In the high-dose group, the 14 tumors were observed in three females: one found dead during week 75 had 5 tumors and two sacrificed at the termination of the study had 9 tumors (3 and 6 per mouse, respectively).

Primary Neoplasms (Tumors) in Male Mice

Difenzoquat (ppm)	0	200	500	1000
Benign tumors	Numerical incidence in males			
Alveolar/bronchiolar adenoma				
Lung	4	6	3/64	3
Cortical adenoma				
Adrenals	2	0/64	2/63	0
Follicular cell adenoma				
Thyroid	0/64	1	0/64	0/64
Hemangioma				
Inguinal lymph node	1/1	-	-	-
Liver	0	0	1/64	0
Skeletal muscle	1/64	0	0/64	0
Spleen	0	0	0/64	1
Hepatocellular adenoma				
Liver	4	0	0/64	1
Interstitial cell tumor				
Testes	1	0	0/64	0
Lipoma				
Skeletal muscle	1/64	0	0/64	0
Malignant tumors	Numerical incidence in males			
Alveolar/bronchiolar carcinoma				
Lung	1	0	1/64	0
Hemangiosarcoma				
Liver	0	2/65	0/64	0
Hepatocellular carcinoma				
Liver	0	0	0/64	1
Malignant fibrous histiocytoma				
Tail	0/1	0/2	1/1	-
Malignant lymphoma				
Lymphatic tissue	0	1	0	0

Continued on next page

Primary Neoplasms (Tumors) in Male Mice - continued

Difenzoquat (ppm)	0	200	500	1000
Malignant tumors	Numerical incidence in males			
Hemangiosarcoma				
Brain	1	0	0/64	0
Sarcoma				
Epididymides	1/1	-	-	-

This table is based on Table 29, pages 231-256, of the submitted report (MRID No. 42800402). Numerical incidence = Number of organs (tissues) affected / Number of organs (tissues) examined. Unless indicated otherwise in the above table, organs (tissues) from all male mice in the study (65/group) were examined.
- Tissue not examined.

Primary Neoplasms (Tumors) in Female Mice

Difenzoquat (ppm)	0	200	500	1000
Benign tumors	Numerical incidence in females			
Adenoma				
Lacrimal gland	-	-	1/1	-
Alveolar/bronchiolar adenoma				
Lung	2	2	1	0
Capsular cell adenoma				
Adrenals	0	1	0	0
Cortical adenoma				
Adrenals	0	1	0	0
Cystadenoma				
Harderian gland	-	1/1	-	-
Endometrial stromal polyp				
Uterus	2	1	1	0
Follicular cell adenoma				
Thyroid	1	0	1	0

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Primary Neoplasms (Tumors) in Female Mice - continued

Difenzoquat (ppm)	0	200	500	1000
Benign tumors	Numerical incidence in females			
Hemangioma				
Liver	3	0	0/64	1
Ovaries	0	0	1/64	0
Spleen	0	1	0	0
Uterus	1	0	1	0
Leiomyoma				
Cervix	0/62	1/64	1/59	0/61
Uterus	1	1	0	2
Odontoma				
Calvarium/skull	1/1	-	0/2	0/1
Malignant tumors	Numerical incidence in females			
Adenocarcinoma				
Skin	0	0	1	0
Alveolar/bronchiolar carcinoma				
Lung	0	1	1	0
Anaplastic sarcoma				
Peritoneum/cavity	0/3	1/2	0/2	-
Hemangiosarcoma				
Spleen	0	0	0	1
Hepatocellular carcinoma				
Liver	1	0	0/64	0
Histiosarcoma				
Uterus	1	3	0	1
Leiomyosarcoma				
Uterus	0	0	0	1
Malignant lymphoma				
Hemic/lymphatic	1	4	3	2
Osteosarcoma				
Skeletal muscle	0	1	0	0/64

Continued on next page

Primary Neoplasms (Tumors) in Female Mice - continued

Difenzoquat (ppm)	0	200	500	1000
Malignant tumors	Numerical incidence in females			
Sarcoma				
Cervix	1/62	0/64	1/59	0/61
Thymic lymphoma				
Thymus	0/59	1/56	0/52	2/56

This table is based on Table 29, pages 231-256, of the submitted report (MRID No. 42800402). Numerical incidence = Number of organs (tissued) affected / Number of organs (tissues) examined. Unless indicated otherwise in the above table, organs (tissues) from all female mice in the study (65/group) were examined.
- Tissue not examined.

The above data (primary neoplasms in males and females) were not analyzed statistically because, according to page 26 of the submitted report, " None of the tumors cited met the criteria for performing statistical analysis " (see Attachment I of this review). It was also stated on page 26 that none of the tumors reported in this study had incidences higher than the testing facility's historical control data. The historical control data were not submitted. However, the incidence of primary neoplasms in males and females was very low and dose-unrelated, and, in most instances, was lower in the high-dose group than in the control group. The most common neoplasm observed in males and females was pulmonary adenoma but, again, the incidence was lower in the high-dose group than in the control and the low-dose and mid-dose groups.

COMMENTS

This study is well planned and, in general, well reported, and it meets the December 24, 1989, EPA ACCEPTANCE CRITERIA. The procedures used in the assignment of mice to groups, diet preparation and analyses, hematology and histopathology have been described. The statistical procedures used have been described and referenced. In each instance, when checking was done, data reported in the summary Volume I were easily found in the volumes containing individual data. However, a few errors were discovered in Table 30 (Neoplasm Incidence by Animal Number and Week of Death) of the submitted report, as follows:

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Page 258: Female no. 3479 from Group 2 (low-dose) was listed in Group I (control group).

Female no. 3560 from Group 3 (mid-dose) was listed in Group 2.

Page 263: Females no. 3750, 3739 and 3749 from Group 4 (high-dose) were listed in Group 3.

The most puzzling observation in this study was the presence of convulsions in all groups, including the controls. As was reported on page 8 of this review, the dose-unrelated convulsions were observed in 29-37% of male mice and in 29-43% of female mice per group. These convulsions were observed during the study weeks 9-78, but their duration and severity were not reported by the testing facility. However, most mice obviously recovered because mortality was low in this study and no reference to convulsions was made in macroscopic observations. Since a high incidence of convulsions is not a normal occurrence in the untreated, healthy animals, some explanation from the author of this report (MRID No. 42800402) is in order. Also, a description of what was regarded as convulsions should be submitted.

The following statements were included in the submission:

1. Statement of No Data Confidentiality Claims.

2. Statement of Compliance with the EPA Pesticide Programs Good Laboratory Practice Standards, 40 CFR 160.

3. Data Flagging Statement (there are no 6a-2 data in this study).

4. Quality Assurance Statement. This study, in life and final report, was inspected 39 times during March 25, 1987 and June 22, 1989, when the final report was reviewed. The inspections were conducted by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc.

This study was submitted in response to the requirements of the 1988 Registration Standard for Difenzoquat Methyl Sulfate. Considering decreases in body weight gain discussed in this review, it appears that the 1000 ppm dose, the highest tested, was high enough to assess the carcinogenic potential of Difenzoquat.

ATTACHMENT I

AVENGE (DIFENZOQUAT)

Page_____ is not included in this copy.

Pages 65 through 67 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.

X 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.

ATTACHMENT II

Chronic Dietary Toxicity and Oncogenicity Study with AC 84,777 in Mice - Most Common Non-neoplastic Findings

Difenzoquat (ppm)	0	200	500	1000	0	200	500	1000
Finding	Number of mice with finding/Number examined							
	Males				Females			
Amyloidosis								
Adrenals	8/65	15/64	11/63	21/65	16/65	13/65	19/65	12/65
Duedenum	4/63	6/58	5/61	11/61	13/61	13/62	15/59	5/62
Heart	6/65	6/65	5/65	14/65	10/65	9/65	15/65	7/65
Ileum	11/63	19/58	13/63	24/63	24/62	19/62	25/60	16/65
Jejunum	5/63	6/55	7/61	11/57	16/61	16/63	15/65	11/62
Kidneys	7/65	12/65	10/64	16/65	17/65	13/65	20/65	9/65
Liver	4/65	6/65	6/64	12/65	7/65	7/65	13/64	2/65
Mesenteric LN	1/63	1/63	0/65	3/64	6/64	5/62	5/64	2/64
Ovaries	-	-	-	-	16/65	7/65	15/64	10/65
Parathyroids	2/54	3/53	4/50	5/57	7/60	7/61	10/51	3/55
Salivary glands	6/65	1/65	5/64	5/65	6/65	3/65	8/65	3/65
Spleen	4/65	7/65	7/64	15/65	8/65	9/65	14/65	6/65
Stomach	1/64	9/64	2/64	4/64	2/64	4/64	5/63	3/65
Thyroid	6/64	10/65	10/64	15/64	11/65	11/65	17/65	9/65
Testes	1/65	2/65	1/64	11/65	-	-	-	-
Involution								
Thymus	40/47	49/50	42/48	47/50	49/59	46/56	42/52	50/56
Subcapsular cell hyperplasia								
Adrenals	8/65	6/64	3/63	5/65	47/65	36/65	34/65	39/65
Cystic endometrial hyperplasia								
Uterus	-	-	-	-	41/65	36/65	41/65	31/65

Continued on next page

Chronic Dietary Toxicity and Oncogenicity Study with AC 84,777 in Mice - Most Common Non-neoplastic Findings --- continued

Difenzoquat (ppm)	0	200	500	1000	0	200	500	1000
Finding	Number of mice with finding/Number examined							
	Males				Females			
Cysts								
Ovaries	-	-	-	-	28/65	39/65	25/64	19/65
Kidneys	2/65	5/65	11/64	5/65	4/65	3/65	3/65	0/65
Mononuclear cell infiltration								
Kidney	21/65	15/65	17/64	19/65	12/65	13/65	17/65	9/65
Liver	7/65	8/65	4/64	1/65	4/65	4/65	7/64	1/65
Salivary glands	9/65	1/65	7/65	6/65	13/65	6/65	11/65	6/65
Inflammation								
Liver	3/65	2/65	1/64	7/65	13/65	12/65	10/64	12/65
Mineralisation								
Brain	4/65	5/65	6/64	2/65	3/65	2/65	3/65	2/65
One lens missing								
Eyes	3/65	3/65	5/64	1/65	6/65	3/65	4/64	6/65
Follicular cysts								
Thyroid	8/64	6/65	3/64	7/64	4/65	7/65	5/65	5/65
Congestion								
Mesenteric LN	10/63	6/63	7/62	6/64	14/64	5/62	11/64	13/64
Pigment								
Mesenteric LN	6/63	2/63	6/62	3/64	10/64	3/62	5/64	5/64

Continued on next page

Chronic Dietary Toxicity and Oncogenicity Study with AC 84,777 in Mice - Most Common Non-neoplastic Findings --- continued

Difenzoquat (ppm)	0	200	500	1000	0	200	500	1000
Finding	Number of mice with finding/Number examined							
	Males				Females			
Lymphoreticular hyperplasia								
Mandib./cer	5/5	5/6	2/3	2/2	1/4	1/5	4/6	0/1
Thymus	3/47	4/50	2/48	3/50	0/59	0/56	0/52	1/56
Ulceration								
Stomach	1/64	1/64	1/64	3/64	3/64	5/64	1/64	3/65
Hyperplasia of glandular mucosa								
Stomach	1/64	1/64	3/64	1/64	3/64	2/64	4/64	4/65

This table is based on Table 29, pages 231-256, of the submitted report (MRID No. 42800402).

AC 84,777 = Difenzoquat methyl sulfate or Difenzoquat

LN = Lymph node

ATTACHMENT III

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Metastatic, Locally Invasive and Other Neoplasms in Male Mice

Difenzoquat (ppm)	0	200	500	1000
Metastatic neoplasms		Numerical incidence		
Malignant fibrous histiocytoma				
Preputial gland	1/3	0/1	0/4	-
Spinal cord	0	0	1	0
Locally invasive neoplasms		Numerical incidence		
Hemangiosarcoma				
Calvarium/skull	1/2	-	0/1	-
Pituitary	1/59	0/63	0/59	0/63
Other neoplasms		Numerical incidence		
Malignant lymphoma				
Liver	0	1	0/64	0
Lung	0	1	0/64	0
Spleen	0	1	0/64	0

This table is based on Table 29, pages 231-256, of the submitted report (MRID No. 42800402).

Numerical incidence = Number of organs (tissues) affected / Number of organs (tissues) examined.

Unless indicated otherwise in the above table, organs (tissues) from all male mice in the study (65/group) were examined.

- Tissue not examined.

Metastatic, Locally Invasive and Other Neoplasms in Female Mice

Difenzoquat (ppm)	0	200	500	1000
Metastatic neoplasms		Numerical incidence		
Carcinoma				
Adrenals	1	0	0	0
Aorta	1	0	0	0
Lung	1	0	0	0
Mediastinal/ bronchial lymph node	1/51	0/57	0/54	0/56
Mesenteric lymph node	1/64	0/62	0/64	0/64
Ovaries	1	0	0/64	0
Pancreas	1	0	0	0
Peritoneum/cavity	1/3	0/2	0/2	-
Spleen	1	0	0	0
Stomach	1/64	0/64	0/63	0
Uterus	1	0	0	0
Hemangiosarcoma				
Liver	0	0	0/64	1
Sarcoma				
Anterior mesenteric/ pancreatic lymph node	1/2	1/3	-	0/2
Bile duct	0	1	0/64	0
Cervix	1/62	0/64	0/59	0/61
Diaphraghm	0	1	0	0
Liver	1	2	0/64	1
Lung	1	1	1	0
Pancreas	1	2	0	0
Renal lymph node	1/6	0/6	0/3	0/1
Urinary bladder	1	0	0/63	0/63
Vaging	1	0/64	0	0/64
Thymic lymphoma				
Anterior mesenteric/ pancreatic lymph node	0/2	0/3	-	1/2
Heart	0	0	0	1
Lung	0	0	0	2
Pancreas	0	0	0	1

Continued on next page

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Metastatic, Locally Invasive and Other Neoplasms in Female Mice - continued

Difenzoquat (ppm)	0	200	500	1000
Locally invasive neoplasms		Numerical incidence		
Thymic lymphoma				
Aorta	0	1	0	2
Mediastinal/ bronchial lymph node	0/51	0/57	0/54	1/56
Pericardial sac	-	1/1	-	-
Pleura/cavity	-	-	-	1/1
Spinal cord	0	1	0	1
Sternum with marrow	0	1/62	0/64	2
Other neoplasms		Numerical incidence		
Malignant lymphoma				
Adrenals	1	2	2	0
Anterior mesenteric/ pancreatic lymph node	1/2	1/3	-	1/1
Axillary/brachial lymph node	0/2	1/1	0/1	-
Cecum	0/64	0/61	0/61	1
Ear	0/2	0/1	1/4	-
Eyes	0	0	1/64	0
Inguinal lymph node	-	2/2	1/1	-
Kidneys	1	1	1	2
Liver	1	3	3/64	1
Lumbar/iliac lymph node	1/9	2/9	1/3	0/2
Lung	1	0	3	0
Mammary gland	0/61	0/60	1/56	0/59
Mandibular/cervical lymph node	1/4	3/5	2/6	1/1
Mediastinal/bronchial lymph node	1/51	3/57	2/54	1/56
Mesenteric lymph node	1/64	3/62	3/64	2/64
Ovaries	1	0	3/64	0
Pancreas	1	3	2	0
Peritoneum/cavity	1/3	1/2	1/2	-
Popliteal lymph node	-	2/2	-	-
Renal lymph node	1/6	4/6	1/3	1/1
Salivary glands	1	1	1	0
Skeletal muscle	1	0	1	0/64
Skin	1	0	1	0
Spinal cord	1	0	1	0
Spleen	1	4	3	1

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Metastatic, Locally Invasive and Other Neoplasms in Female Mice - continued

Difenzoquat (ppm)	0	200	500	1000
Other neoplasms		Numerical incidence		
Malignant lymphoma				
Sternum with marrow	1	0/62	2/64	1
Stomach	1/64	1/64	1/63	1
Thymus	1/59	3/56	3/52	1/56
Thyroid	1	0	1	0
Tongue	1	0	0	0
Urinary bladder	1	2	1/63	0/63
Uterus	1	0	0	0

This table is based on Table 29, pages 231-256, of the submitted report (MRID No. 42800402).

Numerical incidence = Number of organs (tissues) affected / Number of organs (tissues) examined.

Unless indicated otherwise in the above table, organs (tissues) from all female mice in the study (65/group) were examined.

- Tissue not examined.

MRID No.: 42800402
Study No.: HLA 6123-145
Study date: June 23, 1989

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Subdivision F
Guideline Ref. No. 83-2
December 24, 1989

83-2 Oncogenicity in Rats or (Mice)

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 50 animals/sex/group (3 test groups and control group).
3. ✓ Dosing duration is at least 18 months for mice and 24 months for rats.
4. ✓ Number of survivors in any group does not fall below 50% at 15 months for mice, 18 months for rats or 25% at 18 months for mice, 24 months for rats.
5. ‡ ✓ Doses tested include an MTD or limit dose if nontoxic (1,000 mg/kg).
6. • ✓ Doses tested include a NOEL for systematic effects.
7. • ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
8. ✓ Individual daily observations.
9. ✓ Individual body weights.
10. ✓ Individual or cage food consumption.
11. ✓ Individual necropsy of all animals.
12. ✓ Blood smear from 10 animals/sex/dose at 12 and 18 months and termination. Differential count high dose and controls, all other doses if high dose shows pathology.
13. ✓ Histopathology of the following tissues performed on all interim sacrifice animals, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.

✓ aorta	✓ jejunum	✓ peripheral nerve
✓ eyes	✓ bone marrow	✓ kidneys†
✓ caecum	✓ liver†	✓ esophagus
✓ colon	✓ lung	✓ ovaries
✓ duodenum	✓ lymph nodes	___ oviduct
✓ brain†	✓ stomach	✓ pancreas
✓ skin	✓ mammary gland	___ rectum
✓ heart	✓ spleen	✓ spinal cord (3x)
✓ testes†	✓ musculature	✓ thyroid / parathyroids
✓ pituitary	___ epididymis	✓ salivary glands
✓ ileum	✓ adrenals	✓ thymus
✓ trachea	✓ urinary bladder	✓ accessory sex organs; uterus
✓ gall bladder		

† organs to be weighed

‡ The position document entitled "Selection of a Maximum Tolerated Dose (MTD) in Oncogenicity Studies (EPA No. 540/09-88-003) stated EPA's criteria for determining if an oncogenicity study has been adequately performed in terms of doses tested. However OPP is also aware that older oncogenicity studies, upon initial review or re-review, may have been tested at doses lower than the predicted MTD. In the event that such testing appears to be at doses less than the predicted MTD, the Office of Pesticides Program has been reviewing

Criteria marked with a • are supplemental and may not be required for every study.

demonstrated oncogenicity in another species, nearness to the apparent MTD, genotoxic effects, structure-activity factors, absolute value of the highest dose tested and metabolic considerations.

Criteria marked with a • are supplemental and may not be required for every study.