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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Difenzoquat, Toxicology Chapter of the Registration Standard

TOX Chem. No. 363A
TB Project No. 8-0126

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Attached is the Toxicology Chapter of the Registration Standard for Difenzoquat. The following portions of this chapter are available on computer disk. You may obtain a copy from the reviewer.

A. Toxicology Summary
B. Toxicology Profile
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Toxicology Chapter

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A. Toxicology Summary

Difenzoquat (trade name, Avenge) is a selective herbicide used to control wild oats in alfalfa, barley and wheat. Chemically, difenzoquat is a 1,2-dimethyl-3,5-diphenyl-1H-pyrazolium methyl sulfate. The herbicidal mode of action of difenzoquat is not currently understood.

In acute studies, difenzoquat was moderately toxic orally to male rats; slightly toxic dermally to male rabbits and virtually nontoxic to male rats by the inhalation route. Females were not tested in these studies. Toxic signs included dyspnea, prostration and convulsions after oral exposure; lethargy and slight erythema after dermal exposure; and irritation and depression following inhalation exposure.

Difenzoquat was slightly irritating to rabbits' eyes; nonirritating to the intact skin of rabbits; and moderately irritating to the abraded skin. Toxic signs included conjunctival irritation after ocular exposure and severe persistent erythema and edema after application to an abraded skin.

Following a 21-day dermal exposure to difenzoquat, no toxic signs were observed in male rabbits at the 0.5 g/kg level and in female rabbits at the 1.0 g/kg level. Decreased body weight gain was the only toxicity noted in males at the 1.0 g/kg level. However, not all of the currently required parameters were examined in this study.

No compound-related effects were observed in male and female dogs in a 90-day oral feeding study. Systemic NOEL in that study was 2500 ppm (62.5 mg/kg; HDT).

Decreased body weight gain was the only toxic sign noted in both sexes at the 2500 ppm (125 mg/kg) level in a 2-year rat feeding study. In that study, systemic NOEL was 500 ppm (25 mg/kg) for males and females. There are no chronic feeding studies with difenzoquat in nonrodents.

Difenzoquat was negative for oncogenicity at the 5000 ppm (250 mg/kg; HDT) level in the Wistar strain of rats in a 2-year feeding study. The oncogenic potential of difenzoquat in the CD-1 strain of mice could not be determined because too few animals (tissues) were examined microscopically at all dose levels and especially at the 2500 ppm (375 mg/kg) level (HDT) in an 18-month feeding study. An adequate mouse oncogenic study is therefore missing.

Teratogenic, fetotoxic and maternal toxic effects were not

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observed in rabbits at the 100 mg/kg level. However, high mortality and high percentage of animals with resorptions only were observed at the 250 mg/kg level. Difenzoquat was also negative for teratogenicity, fetotoxicity and maternal toxicity in the rat at the 2500 ppm (125 mg/kg; HDT) level. However, the test material was administered in the diet and not by oral intubation. Also, no maternal toxicity was observed at the highest level tested. An adequate teratogenic rat study is therefore missing.

In a 3-generation rat reproduction study, parental NOEL was greater than 2500 ppm (125 mg/kg; HDT) and reproductive/ developmental NOEL was 500 ppm (25 mg/kg). Decreased body weights of male and female pups at weaning in all generations, and decreased body weights of male and female pups at birth in the second and third generation, respectively, were observed at the 2500 ppm level.

Only one mutagenic study (dominant lethal in rats) is currently available, but it is unacceptable. Metabolism studies are missing.

Numerous tolerances on raw agricultural commodities exist for difenzoquat. A Provisional acceptable daily intake (PADI) of 0.08 mg/kg/day was determined from a 2-year rat feeding study.

B. Toxicology Profile

81 Series Acute Toxicity and Irritation Studies

81-1 Acute Oral

Sufficient data are available to show that Technical Difenzoquat has a moderate oral toxicity to mammals (MRID 41883). The acute oral LD_{50} for male rats was 270 (220-340) mg/kg. Toxicity Category II. Females were not tested and a study with female rats is required.

81-2 Acute Dermal

Sufficient data are available to show that Technical Difenzoquat is slightly toxic by the dermal route (MRID 41883). The acute dermal LD_{50} for male rabbits was 3540 (1510-8260) mg/kg. Toxicity Category III. Females were not tested and a study with female rabbits is required.

81-3 Acute Inhalation

Insufficient data are available to assess the toxicity of Technical Difenzoquat by the inhalation route (MRID 45641). The acute LC_{50} for male rats was greater than 298.2 mg/L (1-hr exposure; nominal concentration; Toxicity Category IV), but the size of the particles in the chamber was not determined. Also, female rats were not tested. A study is required.

81-4 Primary Eye Irritation

Sufficient data are available to show that Technical Difenzoquat is a slight eye irritant (Toxicity Category III). At 72 hours after exposure, conjunctival irritation was present in 4 out of 6 rabbits (MRID 41883).

81-5 Primary Dermal Irritation

Sufficient data are available to show that Technical Difenzoquat does not cause dermal irritation when applied on intact skin of rabbits (Toxicity Category IV), but is moderately irritating (Toxicity Category II) when applied on abraded skin (MRID 41883).

81-6 Dermal Sensitization

No dermal sensitization study is available on Technical Difenzoquat. A study is required.

81-7 Acute Delayed Neurotoxicity

No data are available on acute neurotoxic effects of Technical Difenzoquat. This test is required only for compounds which are organophosphate inhibitors of cholinesterase, or related to such inhibitors or metabolites of such inhibitors. Difenzoquat is not an organophosphate, therefore, a study is not required.

82 Series Subchronic Testing

82-1 Subchronic Oral

No data are available on the subchronic oral toxicity of Technical Difenzoquat in rodent. An acceptable chronic study in the rat is available (MRID 36710), therefore, a subchronic study in the rodent is not required. Sufficient data are available to satisfy the data requirement of subchronic oral toxicity studies in a nonrodent.

Young purebred beagle dogs (4-6/sex/group) were dosed with Technical Difenzoquat orally for 90 days at doses of 0, 100, 500 and 2500 ppm in the diet (MRID 37922). No compound-related effects were observed in this study. Systemic NOEL for males and females was 2500 ppm (62.5 mg/kg).

82-2 Subchronic Dermal (21-day)

Insufficient data are available on the subchronic dermal toxicity of Technical Difenzoquat. A study is required.

In the existing study (MRID 41893), New Zealand rabbits (4/sex/group) were dosed with Technical Difenzoquat dermally for 21 days at doses of 0.25, 0.5 and 1.0 g/kg. The test material was applied for six hours a day, five days a week, and one-half of the application sites were abraded. Compound-related effects were decreased body weight gains in the males in the 1.0 g/kg group, NOEL 0.5 g/kg in males, and NOEL > 1.0 g/kg in females. However, application sites were not covered, food consumption not recorded, clinical biochemistry tests not performed, organs not weighed, and only 2 males and 2 females from the high-dose group examined microscopically.

82-3 Subchronic Dermal (90-day)

No data are available on the 90-day subchronic dermal toxicity of Technical Difenzoquat. A study is not required under the present use pattern.

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82-4 Subchronic Inhalation

No data are available on the subchronic inhalation toxicity of Technical Difenzoquat. A study is not required because existing acceptable end-uses should not result in repeated inhalation exposure.

82-5 Subchronic Neurotoxicity

No data are available on the subchronic neurotoxicity of Technical Difenzoquat. Since an acute neurotoxicity study is not required and there is no evidence of neurotoxicity in mammalian species, this study is not required.

83 Series Chronic and Long Term Studies

83-1 Chronic Toxicity

Only a rat chronic feeding study is available. A 1-year feeding study in a nonrodent species is therefore required.

MRID 36710 -- Wistar-derived rats were fed diets containing 0, 100, 500 or 2500/5000 ppm of Technical Difenzoquat for 2 years. After the 30th week, the 2500 ppm level was increased to 5000 ppm; reason for an increase was not given. There were 100 rats per sex in the control group and 60 per sex in each of the treated groups. Interim sacrifice took place at 90 days and included 10 animals of each sex per group.

With the exception of body weights, Difenzoquat had no effect on any of the parameters examined. Compared with the controls, body weight gains were slightly but consistently decreased in the high-dose males and females. These decreases ranged from 4 to 9 percent in the males and from 8 to 16 percent in the females. In the case of males, the decreases were statistically significant ($p = 0.05$) for the test weeks 4, 13 and 26, the only time intervals when statistical significance was calculated. In the case of females, the decreases were statistically significant ($p = 0.05$) only at the test week 26.

(See Section 83-2 for oncogenic effects)

Systemic NOEL = 500 ppm (25 mg/kg, males and females)

Systemic LEL = 2500 ppm (125 mg/kg; decreased body weight gains in males and females)

Classification: Core-Minimum

83-2 Oncogenicity

Sufficient data are available to evaluate the oncogenic potential of Technical Difenzoquat in the rat, but the available mouse oncogenic study is inadequate. A life-time feeding study in the mouse is therefore required.

The chronic study presented above (MRID 36710) is also an acceptable oncogenic study. In that study, Wistar-derived rats, 60 or 100 of each sex per group, were fed diets containing 0, 100, 500 or 2500/5000 ppm of Technical Difenzoquat for 2 years. Ten rats of each sex per group were sacrificed at 90 days (interim sacrifice).

All animals on the study were examined grossly. All tissues (25 or more) were examined microscopically for all animals of the control and the high-dose groups sacrificed at 90 days and at 24 months, and also for all animals sacrificed moribund or found dead in these two groups. For the low-dose and mid-dose groups, at least 8 tissues per animal were examined for 10 animals per sex that were sacrificed at 90 days and 24 months. Tissues examined for these animals were: lungs, heart, kidneys, liver, mammary, adrenals, testes/epididymis. ovary and uterus. Microscopic examinations also included all grossly abnormal sites from all animals, and tissues from all animals found dead or sacrificed moribund.

Predominant neoplastic lesions were observed in the adrenals (cortical adenoma), lungs (reticulum cell sarcoma), pituitary (adenoma), thyroid (adenoma and adenocarcinoma), mammary (adenocarcinoma and fibroadenoma, ovary (adenoma) and uterus (polyps). With the exception of thyroid adenocarcinoma in the mid-dose and high-dose male rats, none of the other tumors were treatment-related. The percent incidence of thyroid adenocarcinoma in the control, low-dose, mid-dose, and high-dose male groups was 4.1, 2.9, 6.3 and 10.2, respectively. The authors of the report regard an increased incidence of thyroid adenocarcinoma in the males as insignificant. According to Dr. Lynnard J. Slaughter, Consulting Pathologist, Toxicology Branch, Hazard Evaluation Division, the historical incidence of thyroid adenocarcinoma in the male Wistar-derived rats is about 19 percent. The incidence of thyroid adenocarcinoma observed in this study is, therefore, within normal limits. Based on decreased body weight gains in the high-dose males and females, it appears that Maximum Tolerated Dose (MTD) was reached.

Difenzoquat was not oncogenic in this study.

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Classification: Core-Minimum

Available data on the mouse (MRID 37923) are inadequate for a meaningful evaluation of the oncogenic potential of Difenzoquat in this species. In this study, CD-1 mice, 60 of each sex per dose level, were fed diets containing 0, 100, 500 or 2500 ppm of Technical Difenzoquat for 18 months. However, too few animals (tissues) were examined microscopically at all dose levels and especially at the 2500 ppm level (HDT).

Classification: Core-Supplementary (Too few parameters were examined)

83-3 Teratogenicity

A valid teratogenic study with rabbits is available. The available teratogenic study with rats is inadequate and a study with this species is required.

MRID 144522 (range-finding study) -- Artificially inseminated New Zealand rabbits, 5/group, were treated with Technical Difenzoquat on gestation days 7 through 17. The test material was administered by gavage at dose levels of 0, 5, 10, 25, 50 or 100 mg/kg of body weight. Cesarean sections were performed on gestation day 29.

Compared with the control group, no compound-related effects were observed at any level of Difenzoquat tested.

Maternal NOEL = > 100 mg/kg/day (HDT)
Developmental NOEL = > 100 mg/kg/day (HDT)

Classification: Core-Supplementary

MRID 144521 -- Artificially inseminated New Zealand rabbits, 18/group, were treated with Technical Difenzoquat on gestation days 7 through 17. The test material was administered by gavage at dose levels of 0, 50, 100, or 250 mg/kg of body weight. Cesarean sections were performed on gestation day 29.

Survival was very low in the high-dose group. Of the 18 inseminated females assigned to each group, 17 (94%),

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2215 (83%), 15 (83%), and 7 (39%) survived until the termination of the study in the control, low-, mid-, and high-dose groups, respectively. Compared with the control group, a high percentage of animals with resorptions only was observed in the high-dose group. The incidence of these resorptions in the control, low-, mid-, and high-dose groups was 6, 17, 15, and 33 percent, respectively. No statistically significant differences were observed in mean body weights or weight changes, uncorrected and corrected for gravid uteri. There were no treatment-related findings at necropsy.

Regarding teratogenic effects, there was a slight and possibly treatment-related increase in the number of fetuses with vertebral central abnormalities in the high-dose group. The fetal percent incidence of this skeletal malformation in the control, low-dose, mid-dose and high-dose groups was 0, 0, 1 and 12, respectively. However, maternal mortality of 61 percent in the 250 mg/kg group resulted in an insufficient number of pups (only 17) to permit a meaningful evaluation of the teratogenic potential of the test material in that (high-dose) group.

Developmental NOEL = > 100 mg/kg
Maternal NOEL = 100 mg/kg
Maternal LEL = 250 mg/kg (HDT; high mortality and high percentage of animals with resorptions only)

Classification: Core-Minimum

The available teratology study in Charles River rats (MRID 37925) is inadequate for reasons stated below and must be repeated.

Developmental NOEL = > 2500 ppm (125 mg/kg; HDT)
Maternal NOEL = > 2500 ppm

Classification: Core-Supplementary (Test material was administered in the diet and not by gavage, and there was no maternal toxicity at the HDT.)

83-4 Reproduction

A valid 3-generation rat reproduction study is available.

MRID 37924 -- Charles River rats were fed diets containing 0, 500 or 2500 ppm of Technical Difenzoquat for three

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successive generations. The feeding of the test material was started 14 weeks (P_1 parents) or 9 weeks (P_2 and P_3 parents) before mating and was continued throughout the mating, gestation and lactation periods.

The only maternal effect observed in all three generations was a decreased body weight gain in the high-dose group during the premating period (the only time when body weights were recorded in this study). Although, compared with the controls, these weight decreases were statistically significant ($p < 0.05$), they were either small (5 or 6%) and probably biologically insignificant or occurred in animals which were smaller (20%) than controls at the start of the treatment, due to reduced weight at weaning.

Male and female pups born in the high-dose group weighed less at weaning in each generation than did those in the control group. The mean weight decreases ranged from 13.9 to 15.5% and were statistically significant ($p < 0.05$). In the low-dose group, the mean weight decreases ranged from 2.1 to 8.7% and all were statistically insignificant. Statistically significant ($p < 0.05$) decreases in the weight of the male pups (7%) and female pups (8.2%) at birth were observed in the $F2_A$ and F_{3A} generations, respectively. These weight decreases were attributed to smaller size of the parents, compared with their respective controls.

Parental NOEL = > 2500 ppm (125 mg/kg; HDT)
Reproductive/Developmental NOEL = 500 ppm (25 mg/kg)
Reproductive/Developmental LEL = 2500 ppm (Decreased body weights of male and female pups at weaning in all generations; decreased body weights of male and female pups at birth in the second and third generation, respectively)

Classification: Core-Minimum

84 Series Mutagenicity

84-2 Mutagenicity Tests.
1. Gene mutation.
2. Chromosomal aberration.
3. Direct DNA damage.
4. Other tests.

Insufficient data are currently available to evaluate the mutagenic potential of Technical Difenzoquat. Studies listed above are required.

The only mutagenic study currently available is a dominant lethal study in which male rats were fed diets containing 0,

500, or 2500 ppm of Technical Difenzoquat for 60 days before they were mated with untreated females (MRID 30577).

The test material was not mutagenic in this study, as judged by the incidence of embryonic mortality, when the females mated with treated males were compared with those mated with untreated males. However, this study was classified as Unacceptable because a positive control was not reported. This classification can be upgraded to Acceptable upon receipt of appropriate positive control data.

85 Series Special Studies

No data are available on the metabolism of Difenzoquat. A study is required.

C. Data Gaps

Difenzoquat is registered for use on food crops and has food tolerances. The following Guideline toxicology studies can be required for this registration:

81-1 Acute Oral Toxicity
81-2 Acute Dermal Toxicity
81-3 Acute Inhalation Toxicity
81-4 Primary Eye Irritation
81-5 Primary Dermal Irritation
81-6 Dermal Sensitization

82-1 Subchronic Oral Dosing in Two Species
82-2 Subchronic Dermal (21-day)

83-1 Chronic Toxicity
83-2 Oncogenicity in Two Species
83-3 Teratogenicity in Two Species
83-4 Reproduction

84-2 Mutagenicity

85-1 Metabolism

Based on this assessment of the toxicology data base for Difenzoquat, the following Guideline toxicology studies have been identified as data gaps and are required:

81-1 Acute Oral Toxicity in One Sex (females)
81-2 Acute Dermal Toxicity in One Sex (females)
81-3 Acute Inhalation Toxicity in One Sex (females)
81-6 Dermal Sensitization

82-2 Subchronic Dermal (21-day)

83-1 Chronic Toxicity in One Species (nonrodent)
83-2 Oncogenicity in One Species (mouse)
83-3 Teratogenicity in One Species (rat)

84-2 Mutagenicity

Gene mutation, chromosomal aberration, and direct DNA damage studies

85-1 Metabolism

D. ADI Reassessment

Because a chronic feeding study in a nonrodent species and a teratology study in the rat are missing, only a PADI for Technical Difenzoquat is currently available.

The PADI for Difenzoquat was based on a 2-year rat feeding study (MRID 36710). Compound-related effects consisted of decreased body weight gains in males and females. The "no observed effect level" (NOEL) in the study was 500 ppm (25 mg/kg). Utilizing a safety factor or 300, the PADI was set at 0.08 mg/kg. This PADI was confirmed by the Toxicology Branch ADI Committee.

The ADI will be made final by an amendment of this Standard.

E. Toxicological Issues

There are currently no toxicological issues. However, because data gaps exist, a final determination of the toxicological issues of Difenzoquat cannot be made in this Standard.

F. Toxicology Summary Tables

Table A
Generic Data Requirements for Difenzoquat

Data Requirement	Composition[1/]	Use Pattern[2/]	Does EPA Have Data To Satisfy Requirements? (Yes, No, or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology					
ACUTE TESTING					
81-1 - Acute Oral - Rat	TGAI	A	Partially	41883	Yes[4/]
81-2 - Acute Dermal	TGAI	A	Partially	41883	Yes[5/]
81-3 - Acute Inhalation - Rat	TGAI	A	Partially	45641	Yes[6/]
81-4 - Eye Irritation - Rabbit	TGAI	A	Yes	41883	No
81-5 - Dermal Irritation - Rabbit	TGAI	A	Yes	--	No
81-6 - Dermal Sensitization - Guinea Pig	TGAI	A	No	--	Yes
81-7 - Acute Delayed Neurotoxicity - Hen	TGAI	A	No	--	No[7/]
SUBCHRONIC TESTING					
82-1 - 90-Day Feeding					
- Rodent	TGAI	A	No	--	No[8/]
- Nonrodent	TGAI	A	Yes	37922	No

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Table A
Generic Data Requirements for Difenzoquat

Data Requirement	Composition[1/]	Use Pattern[2/]	Does EPA Have Data To Satisfy Requirements? (Yes, No, or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology					
SUBCHRONIC TESTING (cont'd)					
82-2 - 21-Day Dermal	TGAI	A	Partially	41893	Yes[9/]
82-3 - 90-Day Dermal	TGAI	A	No	--	No[10/]
82-4 - 90-Day Inhalation	TGAI	A	No	--	No[11/]
82-5 - 90-Day Neurotoxicity	TGAI	A	No	--	No[12/]
CHRONIC TESTING					
83-1 - Chronic Toxicity					
- Rodent	TGAI	A	Yes	36710	No
- Nonrodent	TGAI	A	No	--	Yes[13/]
83-2 - Oncogenicity Study					
- Rat	TGAI	A	Yes	36710	No
- Mouse	TGAI	A	Partially	37923	Yes[14/]
83-3 - Teratogenicity					
- Rat	TGAI	A	Partially	37923	Yes[15/]
- Rabbit	TGAI	A	Yes	144522 144521	No
83-4 - Reproduction	TGAI	A	Yes	37924	No

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Table A
Generic Data Requirements for Difenzoquat

Data Requirement	Composition[1/]	Use Pattern[2/]	Does EPA Have Data To Satisfy Requirements? (Yes, No, or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3/]
§158.135 Toxicology					
MUTAGENIC TESTING					
84-2 - Gene Mutation	TGAI	A	No	--	Yes[16/]
84-2 - Chromosomal Aberration	TGAI	A	No	30577	Yes[17/]
84-2 - Other Mechanisms of Mutagenicity	TGAI	A	No	--	Yes[16/]
SPECIAL TESTING					
85-1 - General Metabolism	PAI or PAIRA	A	No	--	Yes[18/]
85-2 - Domestic Animal Safety	Choice	A	No	--	No[19/]

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Table A
Generic Data Requirements for Difenzoquat

§158.135 Toxicology Footnotes

1/Composition: TGAI Technical Grade Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient, Radiolabelled; Choice = Choice of several test substances determined on a case-by-case basis.
2/The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Nonfood; C = Aquatic, Food Crop; D = Aquatic, Nonfood; E = Greenhouse, Food Crop; F = Greenhouse, Nonfood; G = Forestry; H = Domestic Outdoor; I = Indoor; IP = Industrial Preservative.
3/Unless otherwise specified data must be submitted no later than 6 months after publication of this Standard.
4/The existing study is inadequate because only male rats were tested. A study with female rats is required.
5/The existing study is inadequate because only male rabbits were tested. A study with female rabbits is required.
6/The existing study is inadequate because only male rats were tested and concentration of the test material in the inhalation chamber and the size of particles were not determined. A full study is required.
7/This study is not required because Difenzoquat is not an organophosphate.
8/The acceptable 2-year rat study available fulfills this requirement.
9/The existing study is inadequate because of deficiencies in experimental procedures. A new study must be submitted no later than 7 months after the publication of this Standard.
10/This study is not needed because the existing acceptable end-uses should not result in repeated human skin contact for extended periods.
11/This study is not needed because the existing acceptable end-uses should not result in repeated inhalation exposure.
12/This study is not required because an acute delayed neurotoxicity study is not required.
13/Data must be submitted no later than 42 months after the publication of this Standard.
14/The available study is inadequate. Data for a full study must be submitted no later than 42 months after the publication of this Standard.
15/The existing study has deficiencies in experimental procedures and does not satisfy the regulatory requirement. New data must be submitted no later than 15 months after the publication of this Standard.
16/Data must be submitted no later than 10 months after the publication of this Standard.
17/The existing study can be upgraded from Unacceptable to Acceptable by submitting appropriate positive control data.
18/Data must be submitted no later than 14 months after the publication of this Standard.
19/Considering the use pattern, these data are not required.

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Table B

Product-Specific Data Requirements for Manufacturing-Use Products Containing Difenzoquat

Data Requirement	Composition[1/]	Does EPA Have Data To Satisfy Requirements? (Yes, No, or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[2/]
§158.135 Toxicology				
ACUTE TESTING				
81-1 - Acute Oral - Rat	MP	Partially	41883	Yes[3/]
81-2 - Acute Dermal	MP	Partially	41883	Yes[4/]
81-3 - Acute Inhalation - Rat	MP	Partially	45641	Yes[5/]
81-4 - Primary Eye Irritation - Rabbit	MP	Yes	41883	No
81-5 - Primary Dermal Irritation - Rabbit	MP	Yes	41883	No
81-6 - Dermal Sensitization - Guinea Pig	MP	No	--	Yes

1/Composition: MP = Manufacturing-use product.
2/Unless otherwise specified data must be submitted no later than 6 months after publication of this Standard.
3/The existing study is inadequate because only male rats were tested. A study with female rats is required.
4/The existing study is inadequate because only male rabbits were tested. A study with female rabbits is required.
5/The existing study is inadequate because only male rats were tested, the concentration of the test material in the inhalation chamber, and the size of particles were not determined. A full study is required.

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00045641 DeProspo, J. (1973) Acute Inhalation Study of Avenge 95-S in Rats: Contract No. 120-2059-83. Final rept. (Unpublished study received Nov 14, 1975 under 6F1703; prepared by Affiliated Medica Research, Inc., submitted by American Cyanamid Co., Princeton, N.J.; CDL:094730-D)

00144521 Mackenzie, K. (1984) A Teratology Study with AC 84,777 in Rabbits: Final Report: Study No. 6123-114. Unpublished study prepared by Hazleton Laboratories America, Inc. 61 p.

00144522 Mackenzie, K. (1984) A Range Finding Study with AC 84,777 in Rabbits: Final Report: Study No. 6123-113. Unpublished study prepared by Hazleton Laboratories America, Inc. 50 p.

H. One Liners

Tox. Chem. No. 363A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute Oral - Rat; American Cyanamid Company; No. A-73-38; June 28, 1973; MRID No. 41883	Technical; purity not stated		LD_{50} = 270 mg/kg; males Dyspnea, prostration, convulsions. Only males were used and duration of the observation period was not reported.	II	001266 Supplementary 006658
Acute Dermal - Rabbit; American Cyanamid Company; No. A-73-38; June 28, 1973; MRID No. 41883	Technical; purity not stated		LD_{50} = 3540 mg/kg; males Lethargy and slight erythema; 24-hour exposure Only males were used and duration of the observation period not reported.	III	001266 Supplementary 006658
Acute Inhalation - Rat; Affiliated Med. Res.; No. 120-2059-83; September 13, 1973; MRID No. 45641	Average 95-5 96.4% technical		LC_{50} = > 298.2 mg/L (males only, 1-hour exposure, nominal concentration) No mortality; transient irritaion and mild depression. Size of particles not determined.	IV	001266 Supplementary 006658
Primary Eye Irritation - Rabbit; American Cyanamid Company; No. A-73-38; June 28, 1973; MRID No. 41883	Technical; purity not stated		Moderate conjunctival irritation only. Observation period: 72 hours.	III	001266 Minimum 006658

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Tox. Chem. No. 363A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary Dermal Irritation - Rabbit; American Cyanamid Company; No. A-73-38; June 28, 1973; MRID No. 41883	Technical; purity not stated		Skin reactions by the 72-hour observation period: Intact skin - no erythema or edema. Abraded skin - severe erythema and edema. * For intact skin ** For abraded skin	IV* or II**	001266 Minimum 006658
90-Day Feeding - Dog; Food and Drug Research Laboratories, Inc.; No. 1680; September 28, 1973; MRID No. 37922	Technical; purity: 98.1%		NOEL = 2500 ppm (62.5 mg/kg; HDT; males and females) Levels tested: 0, 100, 500 and 2500 ppm in beagle strain (diet).		001266 Minimum 006658
21-Day Dermal - Rabbit; Biometric Testing, Inc.; No. A-883; September 4, 1973; MRID No. 41893	Technical; purity not stated		NOEL = > 1000 mg/kg (HDT; females) NOEL = 500 mg/kg (males) LEL = 1000 mg/kg (males; decreased body weight gain). Deficiencies in experimental procedures. Levels tested: 0, 250, 500, and 1000 mg/kg in New Zealand strain. Intact and abraded skin.		001266 Supplementary 006658

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Tox. Chem. No. 363A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2-Year Feed/Oncogenic - Rat; Food and Drug Research Laboratories, Inc.; No. 1626; September 19, 1975; MRID No. 36710	Technical; purity: 98.1%		Sys. NOEL = 500 ppm (25 mg/kg); males and females Sys. LEL = 2500 ppm* (125 mg/kg; decreased body weight gains in both sexes). Oncogenic NOEL = > 5000 ppm (250 mg/kg; HDT; males and females) Levels tested: 100, 500, and 2500/5000 ppm in Wistar strain (diet). *High-dose group received 2500 ppm for 30 weeks and 5000 ppm thereafter. Decreased body weight gains were already observed at the 2500 ppm level.		001268 Minimum as a feeding study and as an oncogenic study 006658
18-Month Oncogenic - Mouse; Pharmacopathic Research Laboratories, Inc.; No. 7310 M; February 26, 1975 MRID No. 37923	Technical; purity not stated		Oncogenic NOEL: could not be determined because too few animals (tissues) were examined microscopically at all dose levels and especially at the HDT. Levels tested: 0 (2 controls), 100, 500, and 2500 ppm in CD-1 strain (diet).		Supplementary 006658

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Tox. Chem. No. 363A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology (range-finding) - Rabbit; Hazelton Laboratories America; No. 6123-113; 981-84-101; July 23, 1984 MRID No. 144522	Technical; purity: 96%		Maternal and fetotoxic NOEL = > 100 mg/kg (HDT) Levels tested: 0, 5, 10, 25, 50, and 100 mg/kg in New Zealand strain (gavage).		Supplementary 006658
Teratology - Rabbit; Hazelton Laboratories America; No. 6123-114; 981-84-102; December 14, 1984 MRID No. 144521	Technical; purity: 99.1%		Teratogenic NOEL = > 100 mg/kg (mid-dose)* Fetotoxic NOEL = 100 mg/kg* Maternal NOEL = 100 mg/kg Maternal LEL = 250 mg/kg (HDT; high mortality and high percentage of animals with resorptions only). Levels tested: 0, 50, 100, and 250 mg/kg in New Zealand strain (gavage) *Insufficient pups were available for examination at the HDT.		Minimum 006653
Teratology - Rat; Hazelton Laboratories America; No. 362-150 MRID No. 37925	Technical purity: 98.1%		Teratogenic NOEL = > 2500 ppm (125 mg/kg; HDT) Fetotoxic NOEL = > 2500 ppm Maternal NOEL = > 2500 ppm		001267 Supplementary 006658

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Tox. Chem. No. 363A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - Rat; Hazelton Laboratories America; No. 362-150; July 22, 1974; MRID No. 37925 (cont'd)			Levels tested: 0, 500, and 2500 ppm in Charles River strain (diet). Test material was not administered by gavage and there was no maternal toxicity at the HDT.		
Reproduction (3-gen.) - Rat; Hazelton Laboratories; Americas; No. 362-147 October 14, 1974 MRID No. 37924	Technical; purity not stated		Parental NOEL = > 2500 ppm (125 mg/kg; HDT) Reproductive/Developmental NOEL = 500 ppm (25 mg/kg) Reproductive/Developmental LEL = 2500 ppm (decreased body weights of male and female pups at weaning in all generations; decreased body weights of male and female pups at birth in the second and third generation, respectively.)		001267 001268 Minimum 006658
Mutagenic (Dominant Lethal) - Rat; Food and Drug Research Laboratories, Inc.; No. 2088; September 3, 1974 MRID No. 30577	Technical; purity not stated		Not a mutagen. Levels tested: 0, 500, and 2500 ppm (strain not specified). *Can be upgraded to Acceptable upon receipt of appropriate positive control data.		001267 Unacceptable* 006658

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I. Data Evaluation Reports

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RKL 5/18/88

Reviewed by: Krystyna K. Locke, Toxicologist
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Acute oral (rat)

Tox. Chem. No.: 363A
MRID No.: 41883
Project No.: 8-0126
Doc. No.: 006658

Test Material: Technical Avenge (Sample 73-16); purity not stated

Synonyms: Difenzoquat methyl sulfate; AC 84,777; AC-1786-158

Study Number(s): A-73-38

Sponsor: American Cyanamid Co.

Testing Facility: American Cyanamid Co.

Title of Report: Toxicity Data: Avenge Herbicide, Technical

Author(s): American Cyanamid Co.

Report Issued: June 28, 1973

Conclusions:

LD_{50} = 270 (220-340)[1] mg/kg; males

Toxicity category: II

Classification: Core-Supplementary (only male rats were tested and duration of the observation period was not reported)

This study was originally reviewed by Toxicology Branch in 1974 (see Attachment I). That review is acceptable, but is currently amended by the data listed under Conclusions and by the additional information as follows:

1. The dose levels used were 157, 313, 625 and 1250 mg/kg, but the duration of the observation period was not reported. No

[1] Values in parentheses were not defined and procedure used to calculate LD_{50} not reported.

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reference was also made to the procedures used in assigning animals to groups, source of animals, diets fed, or housing conditions.

2. Animals were weighed initially and at the termination of the study (not stated when). The group mean initial body weights ranged from 137 to 138 g. The survivors (all of the rats in the 157 mg/kg group and 3 rats in the 313 mg/kg group) gained weight. The remaining animals died within 6 to 24 hours after dosing.

3. Necropsy was not performed.

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Attachment I

Rat Oral LD_{50} - American Cyanamid Co. - 6/28/73

The material used in this study was identified as Technical No. 84,777, Sample 73-16. Ten fasted male RH Wistar rats were administered the test material (a 20% w/v aqueous dispersion) in a dosage range of from 157 - 1250 mg/kg.

Results

LD_{50} = 270 (220 - 340 mg/kg). Signs of intoxication included dyspnea, prostration, mild convulsions.

006658

Reviewed by: Krystyna K. Locke, Toxicologist RKL 8/18/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Acute dermal (rabbit)

Tox. Chem. No.: 363A
MRID No.: 41883
Project No.: 8-0126
Doc. No.: 006658

Test Material: Technical Avenge (Sample 73-16); purity not stated

Synonyms: Difenzoquat methyl sulfate; AC 84,777; AC-1786-158

Study Number(s): A-73-38

Sponsor: American Cyanamid Co.

Testing Facility: American Cyanamid Co.

Title of Report: Toxicity Data: Avenge Herbicide, Technical

Author(s): American Cyanamid Co.

Report Issued: June 28, 1973

Conclusions:

LD_{50} = 3540 (1510-8260)[1] mg/kg; males

Toxicity category: III

Classification: Core-Supplementary (Only male rabbits were used and duration of the observation period was not specified)

This study was originally reviewed by Toxicology Branch in 1974 (see Attachment I). That review is acceptable, but is currently amended by the data listed under Conclusions and by the additional information as follows:

1. The dose levels used (625, 1250, 2500, 5000 and 10000 mg/kg of body weight) were applied on intact skin. The strain of animals used and the duration of the observation period were not

[1] Values in parentheses were not defined and procedure used to calculate LD_{50} not reported.

reported. No reference was also made to the procedures used in assigning animals to groups, source of animals, diets fed, or housing conditions.

2. Animals were weighed initially and at the termination of the study (not stated when). The group mean initial body weights ranged from 2.65 to 2.96 kg. The survivors in the 10000 mg/kg group lost weight, whereas the remaining animals gained, in a dose-unrelated manner, a total of 0.08 to 0.2 kg (group mean values).

3. It was not reported if the application sites were washed or wiped at the termination of the exposure, and what procedure was used to evaluate appearance of the application sites.

4. Necropsy of the survivors revealed nothing remarkable; nonsurvivors were not necropsied.

5. Toxic signs and most of the deaths occurred within 6 to 24 hours after exposure.

Attachment I

Rabbit Dermal LD_{50} - American Cyanamid Co. - 6/28/73

The test material was identified as Technical No. 84,777, Sample 73-16. An aqueous paste of the test material was applied to each of five rabbits per level. A range of from 625 to 10,000 mg/kg was tested. Exposure was 24 hours under an impervious cuff.

Results

LD_{50} = 3540 (1510 - 8260 mg/kg). Signs of intoxication included lethargy. No edema and slight erythema were noted.

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Reviewed by: Krystyna K. Locke, Toxicologist KKL 5/18/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Acute inhalation (rat)

Tox. Chem. No.: 363A
MRID No.: 45641
Project No.: 8-0126
Doc. No.: 006658

Test Material: Avenge 95-S (96.4% technical)

Synonyms: Difenzoquat; AC 84,777

Study Number(s): 120-2059-83

Sponsor: American Cyanamid Co.

Testing Facility: Affiliated Medical Research, Inc.

Title of Report: Acute Inhalation Study of Avenge 95-S in Rats

Author(s): DeProspo, J.

Report Issued: September 13, 1973

Conclusions:

LC_{50} = > 298.2 mg/L (males only; 1-hr exposure; nominal concentration)

Toxicity Category: IV

Classification: Core-Supplementary (only males were used; actual concentration of the test material in the chamber and size of the particles were not determined; and body weights were not recorded during the observation period).

This study was originally reviewed in Toxicology Branch in 1974 (see Attachment I). That review is acceptable, but is currently amended by the data listed under Conclusions and by the additional information as follows:

1. The initial weight of the test animals ranged from 186 to 201 g and they were housed individually. Strain of animals and length of the acclimation period were not specified.

2. The test material was aerosolized but the type of exposure (whole-body or head-only) was not indicated. 006658

3. The animals were observed during exposure and for 14 days thereafter.

4. Nothing remarkable was noted at necropsy.

5. The designation of Limit Test does not apply to this study.

Attachment I

Rat Inhalation LC_{50} (95-S) - Affiliated Medical Research Inc. - 9/13/73

The test material was identified as Avenge 95-S, 2282-33 which is 96.4% technical [redacted] This formulation was diluted in water to make a 25% w/v suspension. Six young adult male rats were exposed to a nominal concentration of 298.2 mg/L in a dynamic chamber. Length of exposure was one hour.

Observations and tests included daily physical observation, mortality, and gross autopsy.

Results

LC_{50} = greater than 298.2 mg/L

No mortality occurred. Transient irritation and mild depression were noted.

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5/18/88

Reviewed by: Krystyna K. Locke, Toxicologist KKL
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Primary Dermal Irritation (rabbit)

Tox. Chem. No.: 363A
MRID No.: 41883
Project No.: 8-0126
Doc. No.: 006658

Test Material: Technical Avenge (Sample 73-16); purity not stated

Synonyms: Difenzoquat methyl sulfate; AC 84,777; AC-1786-158

Sponsor: American Cyanamid Co.

Testing Facility: American Cyanamid Co.

Title of Report: Toxicity Data: Avenge Herbicide, Technical

Author(s): American Cyanamid Co.

Report Issued: June 28, 1973

Conclusions:

Skin irritation reactions at 72 hours after 24-hour contact with test material were:

Intact skin: No erythema or edema

Abraded skin: Severe erythema and edema

Toxicity category: Intact skin, IV
Abraded skin, II

Classification: Core-Minimum

This study was originally reviewed by Toxicology Branch in 1974 (see Attachment I). That review is acceptable, but is currently amended by the data listed under Conclusions. It should also be noted that skin reactions were evaluated by the procedure of Draize, John H., Woodward, Geoffrey, and Calvery, Herbert O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes", J. Pharm. & Exp. Ther. 82, 377 (1944).

Attachment I

Rabbit Dermal Irritation - American Cyanamid Co. 6/28/73

The test material was identified as Technical No. 84,777, Sample 73-16. An aqueous paste (0.5 gm/rabbit) of the test material was applied to six rabbits under an impervious patch for 24 hours. Half the test sites were abraded. Observations were made at 24 and 72 hours.

Results

The intact test sites completely recovered from the very slight to moderate erythema and negative to slight edema by 72 hours; the abraded test sites did not show a meaningful recovery from the severe edema and erythema by the 72 hour observation period.

006658

Reviewed by: Krystyna K. Locke, Toxicologist KKL 5/17/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)
Budd 5/23/88

DATA EVALUATION REPORT

Study Type: Primary Eye Irritation (rabbit)

Tox. Chem. No.: 363A
MRID No.: 41883
Project No.: 8-0126
Doc. No.: 006658

Test Material: Technical Avenge (Sample 73-16); purity not stated

Synonyms: Difenzoquat methyl sulfate; AC 84,777; AC-1786-158

Study Number(s): A-73-38

Sponsor: American Cyanamid Co.

Testing Facility: American Cyanamid Co.

Author(s): American Cyanamid Co.

Report Issued: June 28, 1973

Conclusions:

At 72 hours after exposure, moderate conjunctival irritation was present in 4 out of 6 rabbits. Corneal opacity and iritis were not observed.

Toxicity category: III

Classification: Core-Minimum

This study was originally reviewed by Toxicology Branch in 1974 (see Attachment I). That review is acceptable, but is currently amended by the data listed in Conclusions. It should also be noted that ocular reactions were evaluated by the procedure of Draize, John H., Woodward, Geoffrey, and Calvery, Herbert O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes", J. Pharm. & Exp. Ther. 82, 377 (1944).

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Attachment I

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Rabbit Eye Irritation - American Cyanamid Co. - 6/28/73

The test material was identified as Technical No. 84,777, Sample 73-16. One-tenth milliliter was instilled in the eye of each of six rabbits. Observations were made at 24, 48 and 72 hours.

Results

No cornea or iris reactions were noted. Moderate irritation of the conjunctivae was recorded.

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KRL 5/10/88

Reviewed by: Krystyna K. Locke, Toxicologist
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

Budd 5/20/88

DATA EVALUATION REPORT

Study Type: Subchronic oral toxicity (dog): 90 days

Tox. Chem. No.: 363A
Doc. No.: 006658
MRID No.: 37922
Project No.: 8-0126

Test Material: Tech. AC 84777; purity: 98.1%

Synonyms: Difenzoquat; Avenge

Study Number(s): 1680

Sponsor: American Cyanamid Company

Testing Facility: Food and Drug Research Laboratories, Inc.

Title of Report: 90-Day Feeding Study in Dogs with AC 84777

Author(s): Cox, G. E.; Bailey, D. E.; and Morgareidge, K.

Report Issued: September 28, 1973

Conclusions:

NOEL = 2500 ppm (62.5 mg/kg; HDT; males and females)

Classification: Core-Minimum (Blood was not analyzed for electrolytes, albumen, total protein, creatinine and bilirubin, and data were not statistically analyzed)

This study was originally reviewed by Toxicology Branch in 1974, but this one-page review (see Attachment I) contains a few omissions and errors in both the experimental procedures and the results. The correct (or missing) information should be as follows:

Experimental Procedures:

1. There were 6 males and 6 females in the control groups and 4 of each sex in the treated groups.

2. The animals were assigned to groups randomly as detailed in Table 1 (which appears in Appendix I, p. 2).

3. The animals were obtained from the breeding unit of the Food and Drug Research Laboratories (Waverly Division), acclimated for several weeks, and housed singly.

4. Body weights at the initiation of the study ranged from 9.0 to 14.3 kg (males) and from 6.9 to 11.3 kg (females).

5. Moistened Purina Dog Chow (kibbled form) was offered daily to each dog for one hour and then the food was removed from the cage and the amount consumed recorded. It was not reported in the submission how much food was offered. The test material-containing diets were offered on a six day a week basis.

6. Ophthalmological examination was conducted prior to initiation and the termination of the study. All animals were examined.

7. Hematology, clinical chemistry and urinalysis were conducted at the pretreatment weeks 1 and 2, and after 4 and 12 weeks of treatment. All animals were used in these tests. All of the analytical procedures used were referenced. The following clinical biochemistry determinations were not performed: calcium, phosphorus, chloride, sodium, potassium, albumen, total protein, creatinine and total bilirubin.

8. Body weights were recorded at the initiation of the study and weekly thereafter.

9. Liver was also weighed, in addition to the organs listed in the Tox. Branch review.

10. Data were not analyzed statistically.

Results

1. There were no mortalities. Appearance and behavior were normal.

2. Body weight gains of the males and females in all treated groups were comparable with those of the respective controls.

3. Necropsy revealed only occasional and treatment-unrelated reddening of the intestinal mucosa.

4. Microscopic examination revealed slight focal interstitial inflammation of pyramid, medulla and/or cortex of kidneys. These findings, regarded by the authors as infrequent in dogs, do not appear to be treatment-related. The incidence of these findings was as follows:

   Males--3 in control and 1 in mid-dose groups

   Females--1 in each control and low-dose groups.

Comment

Although this study was conducted 14.5 years ago, it is scientifically sound, includes most of the parameters that are currently included in a 90-day non-rodent oral feeding study, and should be accepted as a Core-Minimum study.

Attachment I

90 Day Dog Feeding (Technical AC84,777) Food & Drug Research Lab 9/28/73

Four young purebred beagle dogs of each sex were used per level of 100, 500, and 2500 ppm. The test material was offered in the regular diet on a six day a week basis.

Observations and tests for effects included ophthalmological examination, body weights, hematology at 1, 2, 4 and 12 weeks (RBC & WBC, differential count, hemoglobin, hematocrit, erythrocyte sedimentation rate and reticulocytes), clinical test at 1, 2, 4 and 12 weeks (SGOT, SGPT, SAP, coagulation time, BUN and glucose), and urinalysis. Terminal observations included the absolute and ratio weights of the kidney, heart, testes, adrenals and ovaries; microscopic examination of the following:

adrenals
aorta
bone (rib junction)
bone marrow
brain
cholecyst
epididymis
eye
gonad
heart (with coronary vessels)
intestine
colon
duodenum
ileum
jejunum
kidney
lens
liver
lungs
all gross lesions
lymph node mesenteric
mammary glands
nerve (with muscle)
pancreas
pituitary
prostate
salivary gland
skeletal muscle (with nerve)
spinal cord
spleen
stomach
pyloric
fundus
thymus
thyroid
urocyst
uterus

Results

A slight reduction in body weight gains for the 2500 ppm level animals was evident. All other parameters were within normal biological variations. NEL is 2500 ppm.

006658

Reviewed by: Krystyna K. Locke, Toxicologist KKL 5/18/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head ERB 5/23/88
Section II, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Repeated dose dermal toxicity: 21 days--rabbit

Tox. Chem. No.: 363A

MRID No.: 41893

Project No.: 8-0126

Doc. No.: 006658

Test Material: Technical Grade AC 84,777[1]; purity not stated

Synonyms: Difenzoquat; Avenge

Study Number(s): A-883

Sponsor: American Cyanamid Company

Testing Facility: Biometric Testing, Inc.

Title of Report: Repeated Application Dermal Toxicity Study in Rabbits

Author(s): Goldhamer, R. E.

Report Issued: September 4, 1973

Conclusions:

NOEL = > 1.0 g/kg (HDT; females)

NOEL = 0.5 g/kg (males)

LEL = 1.0 g/kg (males; decreased body weight gain)

Classification: Core-Supplementary (Application sites were not covered; food consumption not recorded; clinical biochemistry tests not performed; organs not weighed; hematology and urinalysis performed on only 2 animals/

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[1] Another material identified only as Final Formulation AC 84,777 was also tested, but this review is concerned only with Technical Grade AC 84,777.

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sex/group; and only 2 males and 2 females from the high-dose group were examined microscopically)

This study was originally reviewed by Toxicology Branch (TB) in 1976 (Attachment I), but several important experimental details are missing and the parameters examined, listed in that review, are inaccurate. Also, the reporting of body weight gains and NOEL is inaccurate. The existing review is therefore amended as follows:

Experimental Procedures

1. According to the author, the test method was essentially that of Draize et al.[2]

2. Adult rabbits of New Zealand strain were used in this study after an acclimation period of one week. The initial body weights of males and females ranged from 2.5 to 3.5 kg. The animals were assigned to groups randomly. Source of the animals and housing conditions were not reported.

3. The test material, a powder, (0, 0.25, 0.50 and 1.0 g/kg of body weight) was moistened with deionized water for application. Restrictive collars were used for prevent ingestion of the applied test material. However, the application sites were left uncovered and the possibility that some of the material fell off after it dried cannot be excluded. Following the termination of each exposure, the sites were blotted with dampened absorbent towelling and then dried.

4. Food intake was not recorded. In fact, no reference at all was made to feeding in the submitted report.

5. Hematology (hemoglobin, hematocrit, RBC count, and total and differential WBC count) and urinalysis were performed initially and at the termination of the study. All controls (4 males and 4 females) and one-half of the treated animals (2 males and 2 females/group) were used in these tests. The same animals were used in the clinical tests and microscopic examination.

6. Tests for blood clotting potential and clinical biochemistry tests were not performed.

7. All animals were necropsied but organs were not weighed.

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[2] Draize, John H., Woodard, Geoffrey, and Calver, Herbert O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes", J. Pharm. & Exp. Ther. 82, 377 (1944).

8. The following tissues were preserved for possible microscopic examination: brain, stomach, bladder, eyes, spleen, pancreas, gonads, lungs, small and large intestines, heart, adrenals, and bone marrow.

9. The following tissues were examined microscopically: kidneys, bile duct, liver, and skin and subcutis. All of the controls and 2 males and 2 females from each of the treated groups were examined.

Results

1. There were no deaths and toxic signs were not observed.

2. The mean body weight gains during the study were as follows:

Technical AC 84,777 (g/kg)	Body weight gains (g) Males	Females
0	580	620
0.25	760	480
0.50	550	520
1.00	310	640

These data were not analyzed statistically but, compared with the controls, a decreased weight gain of 46.4% (in the case of the high-dose males) appears biologically significant.

3. Neoplastic lesions were not observed. Non-neoplastic lesions were observed in the kidneys (calcification, vacuolization, and chronic inflammation), bile duct (chronic inflammation and parasites), and liver (chronic inflammation). The incidence of these lesions was similar in the control and treated groups. However, as was indicated in Experimental Procedures, only one-half of the treated animals was examined microscopically.

Comments

This study can only be accepted as Supplementary because several parameters were not tested at all or were tested in too few animals.

A summary of microscopic findings, as well as individual data, each entitled MICROSCOPY FOR BIOLOGICAL RESEARCH, INC., are poorly reported (inadequately identified and, in some instance, illegible). Also, the title is somewhat puzzling, considering that this study was performed by Biometric Testing, Inc. for American Cyanamid Co.

Attachment I

21 Day Rabbit Dermal - American Cynamid 9/4/73

The two test materials used were identified as Final Formulation AC84,777 and Technical Grade AC84,777. The Final Formulation was tested at 2.0, 1.0 and 0.5 ml/kg. The Technical Grade was tested at 1.0, 0.5 and 0.25 gm/kg. Four rabbits of each sex were used per dosage level. The test material was applied for six hours a day five days a week for three weeks. Half the test sites were abraded.

Observations and tests for effects included mortality, weekly body weights, hematology, urinalysis, gross necropsy, and histological examination of the brain, liver, stomach, bladder, eye, spleen, pancreas, gonad, lung, kidney, small intestine, skin, heart, adrenal, large intestine, bone marrow, kidney, bile duct, and skin.

Results

No effect level is greater than 2.0 ml/kg for the "Final Formulation" and greater than 1.0 gm/kg for Technical Grade.

All of the aforelisted parameters revealed no significant differences between the test and control animals.

Reviewed by: Krystyna K. Locke, Toxicologist
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

KKL 5/10/88
Budd 5/29/88

DATA EVALUATION REPORT

Study Type: Combined chronic toxicity/ oncogenicity (rat)

Tox. Chem. No.: 363A
Doc. No.: 006658
MRID No.: 36710
Project No.: 8-0126

Test Material: AC 84,777; Purity: 98.1%

Synonyms: Avenge; Difenzoquat

Study Number(s): 1626

Sponsor: American Cyanamid Company

Testing Facility: Food and Drug Research Laboratories, Inc.

Title of Report: Chronic Oral Toxicity Study in Rats with AC 84,777

Author(s): Baily, D. E.; Gallo, M. A.; and Cox, G. E.

Report Issued: September 19, 1975

Conclusions:

Systemic NOEL = 500 ppm (25 mg/kg), males and females

Systemic LEL = 2500 ppm* (125 mg/kg; decreased body weight gains in both sexes)

Oncogenic NOEL = > 5000 ppm (250 mg/kg; HDT, males and females)

Core classification: Minimum as a chronic feeding study
Minimum as an oncogenic study

*The high-dose group was fed diets containing 2500 ppm of the test material for 30 weeks and 5000 ppm thereafter. No reason was given for the increase, but decreased body weight gains were already observed at the 2500 ppm level.

This study was originally reviewed by Toxicology Branch in 1976, but that review (Attachment I) is too superficial by current standards. Also, no reference was made to the oncogenic

part of this study. This study was, therefore, currently fully evaluated and core-classified.

Experimental Procedures:

Test Animals

FDRL, Wistar-derived rats (initial body weights: 50-51 g) were used. Source of animals was not specified.

Study Design

The dose levels used were 0, 100, 500 or 2500/5000 ppm in the diet. After the 30th week, the 2500 ppm level was increased to 5000 ppm; reason for an increase was not given. There were 100 rats per sex in the control group and 60 per sex in each of the treated groups. Animals were assigned to groups randomly and were housed singly. Interim sacrifice took place at 90 days and included 10 animals/sex/group. Duration of the study was 104 weeks

Diet

Purina Laboratory Chow and water were fed ad libitum. Preparation and storage of the experimental diets, and analyses for stability and concentration of the test compound were not specified.

Observation

Animals were observed daily for toxic signs and mortality.

Body Weight and Food Intake

These were recorded weekly for the first 12 weeks and monthly thereafter. An analysis of variance was conducted on body weight data for the 4, 13, and 26 week periods. Efficiency of food utilization (EFU) was also calculated for the first 12 test weeks.

Ophthalmological Examination

This examination was performed on all rats initially, at 3 months prior to interim sacrifice, and at the termination of the study.

Clinical Chemistry

These tests (see Attachment I) were performed on 6 rats/sex/group at 3, 6, 12, and 24 months. Hematology and urinalysis were also performed at 18 months.

Gross Pathological Examination

All animals on the study were examined.

Microscopic Examination

All tissues (25 or more; see Attachment I) were examined for all animals of the control and the high-dose groups sacrificed at 90 days and at 24 months, and also for all animals sacrificed moribund or found dead in these two groups. For the low-dose and mid-dose groups, at least 8 tissues per animal were examined for 10 animals per sex that were sacrificed at 90 days and 24 months (see Attachment I). Microscopic examinations also included all grossly abnormal sites from all animals, and tissues from all animals found dead or sacrificed moribund.

Organ Weights

At the interim and final sacrifices, organ weights and organ/body weight ratios were determined for all animals. (Organs that were weighed are listed in Attachment I).

Statistics

With the exception of body weights and food intake, none of the data were apparently statistically analyzed.

Results:

Toxic Signs

General appearance and behavior were comparable for the control and treated groups. Randomly occurring, palpable, subcutaneous mammary masses were observed in animals from all four groups.

Survival

Mortality was unaffected by the test material. Excluding the interim sacrifices, the mortality rate in the control, low-dose, mid-dose, and high-dose males was 69, 68, 60 and 56 percent, respectively. The corresponding rates for the female groups were 50, 50, 26, and 30 percent, respectively.

Body Weight Gains

Compared with the controls, body weight gains were slightly but consistently decreased in the high-dose males and females. These decreases ranged from 4 to 9 percent in the males and from 8 to 16 percent in the females. In the case of males, the decreases were statistically significant ($p = 0.05$) for the test weeks 4, 13, and 26, the only time intervals when statistical

significance was calculated. In the case of females, the decreases were statistically significant only at the test week 26.

Food Consumption and Utilization (EFU)

With the exception of a slight decrease (4%) in the EFU (grams of weight gained/100 g of food eaten) in the high-dose group, the test material had no effect on both food consumption and EFU.

Parameters Unaffected by Treatment with AC 84,777

The test material had no effect on ophthalmoscopic findings, hematology, clinical chemistry, urinalysis, organ weights and organ/body weight ratios.

Gross Pathology

These data were reported mostly for individual animals. A comment was also made in the summary of data that less gross pathology was found in the high-dose test animals than in the controls. Dose-unrelated gross findings were observed mostly in the lungs (collapsed, abscessed, mottled, pale, puffy, firm and/or nodular); pituitary (enlarged and masses); adrenals (enlarged); liver (mottled); thyroid (enlarged); kidneys (granular, fluid-filled); and testes (discolored and atrophied).

Non-neoplastic Findings

Predominant but mostly dose-unrelated non-neoplastic changes were observed in the adrenals (hypervolemia, peliosis, vascular dilatation and cortical and medullary vacuolation); heart (fibrosis); kidneys (calcification, casts, epithelial hyperplasia, fibrosis, polyposis, and dilatation of the renal pelvis; liver (vacuolation); lungs (bronchitis, fibrosis, chronic interstitial inflammation, macrophage aggregates, pneumonitis, and peribronchial lymphoid hyperplasia; pancreas (fibrosis and hyperplasia of islet cells; pituitary (congestion, cysts, cystic spaces hyperplasia); spleen (hematopoiesis and pigment); ovaries (cysts, cystic follicles, and interstitial gland formation); uterus (dilatation); and testes (atrophy and fibrosis).

There was a dose-related increased incidence of nodular vacuolation of the adrenal cortex in the females. The percent incidence of this lesion in the control, low- , mid-, and high-dose female groups was 0, 11.1, 18.4 and 21.7, respectively. The corresponding values for the male groups were 18.6, 9.2,0 and 1.7 percent, respectively.

Hyperplasia of the parathyroid was another predominant

lesion. The numerical incidence of this lesion for the control, low-, mid-, and high-dose male groups was 10, 8, 6, and 14, respectively. The corresponding values for the female groups were 20, 5, 2, and 5, respectively. Because the numbers of tissues examined were not reported for parathyroids, the percent incidence cannot be calculated.

Neoplastic Findings

With the exception of tumors summarized below, most of the remaining tumors were single incidences observed in one or two groups, males or females.

Predominant Tumors Diagnosed Microscopically in Rat

AC 84,777 (ppm) Organ and Tumor	0	100	500	5000[1]	0	100	500	5000[1]
	Males				Females			
	Percent Incidence							
Adrenals								
Adenoma of corte[2]	14.4	1.8	11.8	15.2	17.3	26.7	21.0	15.0
Lungs								
Sarcoma (reticulum cell)	21.6	14.5	25.5	24.1	19.2	11.1	7.9	10.3
Pituitary								
Adenoma (chromophobe)	28.7	21.2	33.3	7.4	17.2	26.1	63.1	23.6
Hyperplasia[3]	10.6	9.1	7.4	7.4	11.8	21.7	0.0	9.1
Thyroid								
Adenocarcinoma	4.1	2.9	6.3	10.2	2.1	4.0	11.8	3.6
Adenoma	3.1	2.9	3.1	1.7	1.0	0.0	0.0	1.8
Mammary								
Adenocarcinoma	-	-	-	-	3.1	3.8	11.5	0.0
Fibroadenoma	-	-	-	-	12.3	15.4	7.7	5.4
Ovary								
Adenoma	-	-	-	-	22.2	23.2	22.2	20.7
Uterus								
Polyps	-	-	-	-	8.2	7.3	12.1	6.7

[1] Animals in this group were fed diets containing 2500 ppm of the test material for 30 weeks and 5000 ppm thereafter.

[2] According to the authors of this study, the rather high incidence of cortical adenoma in these animals is largely a result of the choice of nomenclature, cortical adenoma being used to designate small foci of slightly enlarged vacuolated cortical cells if the vascular flow pattern and presence of peripheral compression give an appearance of nodularity.

[3] Both chromophobe adenoma and hyperplasia were listed under the heading Tumors and Proliferation (page 139 of the submission). A comment was also made in the Results and Discussion section of the report that, in this study, chromophobe adenoma of the anterior lobe was defined as hyperplasia causing peripheral compression.

With the exception of thyroid adenocarcinoma in the mid-dose and high-dose male rats, none of the other tumors appear treatment-related. The authors of this report regard an increased incidence of thyroid adenocarcinoma in the males as insignificant. According to Dr. Lynnard J. Slaughter, Consulting Pathologist, Toxicology Branch, Hazard Evaluation Division, the historical incidence of thyroid adenocarcinoma in the male and female Wistar-derived rats is about 19 percent. The incidence of thyroid adenocarcinoma observed in this study is, therefore, within normal limits. According to Dr. Slaughter, the following increased incidences of tumors, listed in the above table, are also within normal limits for this strain of rats: adrenal cortical adenoma in low-dose females; pituitary adenoma in mid-dose males and females; pituitary hyperplasia in low-dose females; and mammary adenocarcinoma and uterine polyps in mid-dose females.

Maximum Tolerated Dose (MTD)

Based on decreased body weight gains in the high-dose males and females, it appears that MTD was reached. Although decreases in body weight gains were small (4 to 9% in males and 8 to 16% in females, compared with the controls), they were statistically significant ($p = 0.05$) and persisted throughout the study.

Comments

Although this study is about 13 years old and not all of the parameters were examined according to the currently accepted standards (for example, only 6 rats/sex/group were used for the hematology and clinical chemistry analyses; blood electrolytes were not determined; and quality assurance inspections were not performed), enough of the important and scientifically sound data are available to accept this study as a valid chronic feeding/oncogenic study.

Systemic NOEL = 500 ppm (25 mg/kg, males and females)

Systemic LEL = 2500 ppm (125 mg/kg; decreased body weight gains in males and females)

Oncogenic NOEL = > 5000 ppm (250 mg/kg; HDT; males and females)

Core Classification: Minimum for each chronic feeding and oncogenic study.

Attachment I

2 Year Rat Feeding - Food and Drug Research Lab - 9/19/75

The material tested was identified as AC 84777 (Tech), Lot No. AC-1786-158; (98.1%). This material was fed to 60 rats of each sex per level of 100, 500, or 2500/5000 ppm. The 2500 ppm level was changed to 5000 ppm after the 30th week.

Observations and tests for effects included body weights, food consumption, opthalmoscopic examination, interim sacrice at day 90, and the following laboratory test:

hematocrit	glucose
hemoglobin	BUN
RBC	SGPT
WBC	SAP
differential count	urinalysis

Terminal studies included a histopathological examination of the following tissues from all rats of the control and high levels:

Brain	Large Intestine
Pituitary	Mesenteric Lymph Node
Eye	Urinary Bladder
Thyroids	Mammary Gland
Heart	Testes/Epididymis
Lung	Prostate
Liver	Ovary/Uterus
Spleen	Bone Marrow
Kidneys	Spinal Cord
Adrenals	Skeletal Muscle
Stomach	Rib Junction
Pancreas	Sciatic Nerve
Aorta	Tissue Masses
Small Intestine	All Lesions

The following tissues were also examined from 10 rats of each sex from each level:

lung	adrenals
heart	testes/epididymis
kidneys	ovary
liver	uterus
mammary	lesions

Terminal studies also included organ weights of the following organs from all animals:

Thyroids	adrenals
Heart	Testes/epididymis
Liver	Ovary
Spleen	Uterus
Kidneys	

Results: 2500/5000 ppm level - body weight gain was depressed for both sexes at the 52, 78 and 104 week periods.

500 ppm level - normal biological variations.

100 ppm level - normal biological variations.

Conclusion: The no effect has to be established at 500 ppm for this study due to the depressed body weight gain at the high level. This effect is not considered to be of a severe nature but rather a consistance one during a significant part of the study.

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Secondary reviewer: Edwin R. Budd, Section Head
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DATA EVALUATION REPORT

Study Type: Oncogenicity (mouse)

Tox. Chem. No.: 363A
Doc. No.: 006658
MRID No.: 37923
Project No.: 8-0126

Test Material: AC 84777; purity not stated

Synonyms: Difenzoquat, Avenge

Study Number(s): 7310M

Sponsor: American Cyanamid Company.

Testing Facility: Pharmacopathic Research Laboratories, Inc. Laurel, MD

Title of Report: Eighteen Month Carcinogenicity Study on AC 84777

Author(s): Tegeris, A. S. and Underwood, P. C.

Report Issued: February 26, 1975

Conclusions:

Oncogenic NOEL : Could not be determined because too few animals (tissues) were examined microscopically at all dose levels and especially at the 2500 ppm level (HDT).

Classification: Core-Supplementary (Too few parameters were examined).

This study was originally reviewed by Toxicology Branch (TB) in 1976, but the one-page review is too superficial (see Attachment I), implies that this study is an acceptable oncogenic study (which is incorrect) and also contains errors.

Briefly, this study was performed as follows:

1. CD-1 mice, 60 of each sex per dose level (and not 120 as stated in TB review) were fed diets (Charles River 19RF Meal) containing 0 (control 1), 0 (control 2), 100, 500, or 2500 ppm of

the test material. No reason was given for using two control groups.

2. The animals were housed 10/sex/ cage; nothing was said about the identification of animals.

3. At 6 and 12 test months, 5 males and 5 females from each group were sacrificed (interim sacrifices).

4. The following parameters were examined:
   a. Body weights -- were recorded initially and every 2 weeks thereafter for the first three months;
   b. Mortality rate and causes of death;
   c. Gross necropsy -- was performed on all animals in the study; and
   d. Histopathology. According to the submitted repor , full microscopic examination was performed on the following animals:
      -All animals sacrificed at 6 and 12 test months;
      -All animals with tumors or gross lesions detected at necropsy; and
      -Selected (how?) animals at the termination of the study (10 males and 10 females from each of the control groups, and from the high-dose group).

The following parameters were not examined:

1. Palpation for masses;
2. Body weights during the entire study;
3. Food consumption;
4. Differential blood count;
5. Organ weights;
6. Microscopic examination of tissues from all animals in the control and high-dose groups;
7. Lung, liver and kidneys were not examined in all animals; and
8. Data were not analyzed statistically.

Reported results:

1. Regarding cageside observations, fighting was reported but no mention was made of any toxic signs.

2. At the termination of the study, the survival rates (corrected for interim sacrifices) were 64, 62, 36, 78, and 50 percent for the 0, 0, 100, 500, and 2500 ppm male groups, respectively. The corresponding survival rates for the female groups were 68, 68, 72, 74, and 74 percent, respectively.

3. At the end of the first three months, high-dose males and females gained about 20 percent and 14 percent, respectively, less weight than did the combined control groups for each sex.

4. The predominant causes of death in all groups were chronic renal disease, pulmonary edema and/or hemorrhage, respiratory disease, and leukemia. In many instances, causes of death could not be determined.

5. All of the animals were examined grossly. Based on the individual data, the following animals were examined microscopically:

	Number of animals examined			
Test group	Males		Females	
	Nonsurvivors	Sacrificed	Nonsurvivors	Sacrificed
Control 1	12	7	8	12
Control 2	13	8	10	8
100 ppm	15	5	6	12
500 ppm	10	8	6	11
2500 ppm	13	1	3	3

In most instances, only tumors observed at necropsy and other gross lesions were examined microscopically. In most instances, only 2 to 6 organs (tissues) per animal were examined. Full microscopic examination was performed only on one or two animals per group.

It should be noted that in Table 4 of the submission (a part of which was reproduced in TB review) neither the number of tissues nor the number of animals examined was reported.

Most of the male and female survivors that were examined microscopically were sacrificed on test days 557-561 (about 18 months) and were probably scheduled sacrifices. Other survivors, examined microscopically, were sacrificed as shown on the next page:

Test group	Number examined		Day sacrificed
	Males	Females	
Control 1	--	1	181[1]
500 ppm	1	3	181
Control 1	1	1	375[2]
Control 2	1	3	375
100 ppm	1	1	375
500 ppm	1	1	375
2500 ppm	1	1	375
Control 1	--	1	499[3]

Yet, it was stated in the submitted report that all of the animals (5/sex/group), killed at 6 and 12 test months, were examined microscopically.

6. Most of the pathology reports are dated February 22, 23, or 25, 1975, that is, one to four days before the date of the study report (February 26, 1975). Also, one pathology report (for a control male that died after 516 days) is dated February 28, 1975, or two days after the date of the study report.

Conclusions:

The authors of this study conclude that: "The incidence of tumors in the mice exposed to various levels of AC 84777 for eighteen months is so low it makes it quite obvious that AC 84777, under the conditions of this experiment, is not carcinogenic to mice." Actually, too few animals (and tissues) were examined microscopically to conclude if AC 84777 is oncogenic. Also, too few parameters were examined (and none evaluated statistically) to determine whether or not a MTD was reached. This study can, therefore, be accepted only as Supplementary data.

---

[1] About 6 months.

[2] About 12 months.

[3] About 16 months.

Attachment I

006653

18 Month Mice Carcinogenic - Pharmacopathics Research Lab - 2/26/75

The material tested was identified as AC 84777. This material was fed to 120 CD-1 outbred albino mice of each sex per level of 0, 100, 500 and 2500 ppm.

Observations and tests for effects included body weights, interim sacrifice at six months and twelve months and mortality.

Terminal studies included a histopathological examination of all tumors and a complete examination of tissues from ten mice of each sex of the high dose group and control group.

Results: As is evident from the following chart, no carcinogenic activity was detected in this study:

Diagnosis	Male				Female			
	0 PPM	100 PPM	500 PPM	2500 PPM	0 PPM	100 PPM	500 PPM	2500 PPM
VASCULITIS	3							
TUMORS, BENIGN								
ADENOMA								
PULMONARY	6		1					
THYROID								1
CYSTADENOMA, OVARY							1	
FIBROMA	1							
HEMANGIOMA, CAVERNOUS, UTERINE							1	
LUTEOMA OF OVARY						1		
TUMORS, MALIGNANT								
CARCINOMA								
ADENOCARCINOMA								
HEPATOMA	3	1					1	
MAMMARY GLANDS						1	1	1
PANCREAS	1							

DIAGNOSIS	Male				Female			
	0 PPM	100 PPM	500 PPM	2500 PPM	0 PPM	100 PPM	500 PPM	2500 PPM
SARCOMA								
FIBROSARCOMA, UTERUS								1
LYMPHOSARCOMA, TESTIS			1					
RETICULUM CELL	1				1			
LEUKEMIA								
LYMPHOCYTIC, CHRONIC	4		2		6	2		
MYELOGENOUS, ACUTE					1			
THYMOMA			1					
METASTASIS								
LUNG					1			

006653

The other test parameters did not indicate a significant difference between the test and control findings.

The NEL = 2500 ppm

006658

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Secondary reviewer: Edwin R. Budd
Section II, Tox. Branch (TS-769C) ERB 5/19/88

DATA EVALUATION REPORT

Study Type: Teratology -- rat

Tox. Chem. No.: 363A
Doc. No.: 006658
MRID No.: 37925
Project No.: 8-0126

Test Material: Avenge; Purity: 98.1%; Lot No. AC-1786-158

Synonyms: AC 84,777; Difenzoquat methyl sulfate

Study Number(s): 362-150

Sponsor: American Cyanamid Company

Testing Facility: Hazelton Laboratories, Inc.,
Vienna, VA

Title of Report: Teratology Study in Rats

Author(s): Reno, F. E.

Report Issued: July 22, 1974

Conclusions:

The test material was negative for teratogenicity, fetotoxicity and maternal toxicity in this study.

Teratogenic NOEL = > 2500 ppm (125 mg/kg; HDT)

Fetotoxic NOEL = > 2500 ppm

Maternal NOEL = > 2500 ppm

Core classification: Supplementary (Test material was administered in the diet and not by gavage, and there was no maternal toxicity at the HDT.)

Experimental Procedures:

Mated Charles River female rats (initial body weights: 191-263 g), 20/dose level, were fed AC 84,777 in the diet from gestation day (gd) 6 through gd 15; cesarean sections were performed on gd 19; and all animals were examined

006659

macroscopically. The levels of AC 84,777 fed were 0, 500 or 2500 ppm. The following observations were recorded: number and location of implantation sites; live and dead fetuses; resorption sites; corpora lutea; and sex, weight, and length (crown-rump) of the fetuses.

After external examination, about one-third of the fetuses from each litter were fixed in Bouin's solution and evaluated for visceral changes by the procedure of Wilson and Warkany (1965)[1]. Gross sections of nasal, orbital, cervical, thoracic and abdominal regions were examined for abnormalities under the dissecting microscope.

The remaining two-thirds of the fetuses from each litter were eviscerated, fixed in ethanol, and stained with alizarin red. The skeleton was then evaluated for relative differences in size, location, abnormalities in bone structure, and degree of ossification.

The animals were:

1. Obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts;

2. Assigned randomly to groups;

3. Fed unrestricted amounts of Purina Laboratory Chow (without AC 84,777 prior to gd 6 and after gd 15) and water;

4. Observed daily for toxic signs and mortality; and

5. Weighed on gd 0, 6, 11, 15 and 19.

Food intake was recorded on gd 6, 11, 15 and 19.

Statistical analysis of group mean body weights, food consumption and cesarean data was performed by the t-test at the < 5.0% probability level (W. J. Dixon and F. J. Massey, Introduction to Statistical Analysis, pp. 123-124, McGraw Hill, 1957).

Results

Maternal Data

[1]Wilson, J. G. and Warkany, J., Teratology: Principles and Techniques. 1st ed. p. 26, The University of Chicago Press. Chicago, 1965.

None of the dams died during the study and toxic signs were not observed. The hunched appearance noted occasionally in 2, 4, and 3 animals from the control, low-dose, and high-dose groups, respectively, lasted for only one day each time and did not appear to be treatment-related.

The test material had no effect on pregnancy rates and body weight gains. The pregnancy rates were as follows:

	Controls	Test Material	
		500 ppm	2500 ppm
Animals mated	20	20	20
Animals pregnant	18	13	17
Pregnancy rate (%)	90	65	85

Compared with the control and the high-dose groups, the decreased pregnancy rate in the low-dose group does not appear to be treatment-related. However, uteri that appeared non-gravid were not fixed in a 10% solution of ammonium sulfide to confirm pregnancy status.

The mean body weight gains (uncorrected for gravid uteri) during gd 0 through 19 were 142, 152 and 135 g in the control, low-dose and high-dose groups, respectively.

Compared with the controls, the mean food consumption was statistically significantly higher ($p < 0.05$) in the low-dose group on gd 6 (9%) and gd 19 (11%), and in the high-dose group on gd 19 (9%), but these increases did not appear to be biologically significant.

At necropsy, all females in the control and high-dose groups appeared normal, as did those in the low-dose groups with the exception of two females in which the uterus was distended with clear fluid.

Litter Data

Treatment-related external abnormalities were not observed. There were 3 small, 3 pale, 1 with blue marks and 1 with displaced internal organs fetuses in the high-dose group, but the incidence of these abnormalities was similar in the control groups, and none were observed in the low-dose group. There were no dead fetuses in any of the groups.

The test material had no effect on the mean number of

implantation sites, ovarian corpora lutea, resorption sites and live fetuses, or on the mean fetal weights and lengths. Statistically significant ($p<0.05$) increases in the number of implantation sites (20%) and live fetuses (19%) in the high-dose group were not considered compound-related.

The test material had no effect on the visceral and skeletal development of the fetuses. The numbers of fetuses examined for visceral (and skeletal) abnormalities were 51(100), 38(90) and 55(132) in the control, low-dose and high-dose groups, respectively.

In conclusion, AC 84,777 was negative for teratogenicity, fetotoxicity and maternal toxicity in this study.

NOEL (teratogenic, fetotoxic and maternal) = > 2500 ppm (125 mg/kg of body weight; HDT)

Core classification: Supplementary. The test material was administered in the diet and not by oral intubation as is currently required. Also, the highest dose level tested produced no maternal toxicity.

Because this study was conducted some 14 years ago, a quality assurance statement is missing.

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Secondary Reviewer: Edwin R. Budd, Section Head
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006658

DATA EVALUATION REPORT

Study Type: Teratology (Range-Finding) - Rabbit

TOX Chem No.: 363A
MRID No.: 144522
Project No.: 8-0126

Doc. No: 006658

Test Material: Avenge; Purity: 96%

Synonyms: Difenzoquat Methyl Sulfate; AC 84,777

Study Number(s): 6123-113; 981-84-101

Sponsor: American Cyanamid Company

Testing Facility: Hazelton Laboratories America, Inc.
Madison, WI

Title of Report: A Range-Finding Study With AC 84,777 in Rabbits

Author(s): Karen M. MacKenzie

Report Issued: July 23, 1984

Conclusions:

Difenzoquat (AC 84,777) was neither maternally toxic nor fetotoxic in this study.

Maternal and fetotoxic NOEL = > 100 mg/kg (HDT).

Core Classification:

Supplementary (it is a range-finding study).

Experimental Procedures:

Artificially inseminated New Zealand rabbits (age: 5 to 6 months), five animals/group, were treated with 0, 5, 10, 25, 50, or 100 mg AC 84,777/kg of body weight on gestation days (gd) 7 to 19. The compound was administered by gavage as a solution in distilled water. Immediately prior to insemination, each doe was injected with chorionic gonadotropin solution (250 IU in 0.25 mL 0.9% saline) via the ear vein. Cesarean sections were performed on gd 29 and all dams were examined macroscopically. The reproductive tract was evaluated for the following:

- Number and location of live and dead fetuses;

- o Early and late resorptions;
- o Empty sites and implantation scars;
- o Unusual coloration and variations in amniotic fluid or placentae; and
- o Any other abnormalities.

Uteri that appeared nongravid were opened and placed in a 10% solution of ammonium sulfide to confirm pregnancy status.

The animals were:

- o Obtained from Hazelton Dutchland, Inc., Denver, PA;
- o Acclimated for 18 days;
- o Assigned randomly to test groups;
- o Housed individually;
- o Identified by numbered metal ear tags;
- o Fed unrestricted amounts of food (Purina Certified Rabbit Chow 5322) and water;
- o Observed twice daily for toxic signs; and
- o Weighed on gd 0, 7, 10, 14, 20, 24, and 29.

Results:

Maternal Data

Survival

Survival was 100 percent in the 0, 5, 10, and 50 mg/kg groups, and 80 and 60 percent, respectively, in the 25 and 100 mg/kg groups. The deaths in these two groups occurred during gd 0 to 7 and were caused by intubation errors.

Antemortem Observations

These included only one incident of diarrhea each in the 0 and 5 mg/kg groups, and reddish-brown urine in one animal in the 50 mg/kg group. Rhinorrhea and/or convulsions were observed in the animals that were injured by intubation.

Body Weights

There were no treatment-related effects on mean maternal body weights or weight changes. During gd 0 to 29, animals in the 0, 5, 10, 25, 50, and 100 mg/kg groups gained 0.40, 0.51, 0.47, 0.50, 0.36, and 0.41 kg, respectively.

Postmortem Observations

No compound-related abnormalities were observed in animals that died accidentally, and all had observable implants.

At scheduled sacrifice (cesarean section), ascites (commonly seen in pregnant rabbits according to this report) was observed in one animal each in the 0, 25, 50, and 100 mg/kg groups, and three animals in the 5 mg/kg group. A pale and mottled liver was observed in one animal in each of the 0 and 100 mg/kg groups, and three animals in the 50 mg/kg group. In addition to a pale and mottled liver, the animal in the 100 mg/kg group had pale kidneys.

Litter Data

Pregnancy rates in the 0, 5, 10, 25, 50, and 100 mg/kg groups were 60, 100, 80, and 100 percent, respectively.

All animals examined at scheduled cesarean section had viable litters; there were no dead fetuses in any litters. The number of corpora lutea and implants, and implantation efficiency, and the number and percent of live and resorbed fetuses were comparable among all groups. (For details, see Attachment I.)

Maternal NOEL = > 100 mg/kg/day (HDT)
Fetotoxic NOEL = > 100 mg/kg/day (HDT)

Core Classification: Supplementary (range-finding study)

A Quality Assurance Statement, dated July 23, 1984 was included in the report.

Attachment

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Attachment I

Study No. 6123-113

Table 5

Summary of Litter Data from Cesarean Sections

	AC 84,777 (mg/kg)					
	0	5	10	25	50	100
Dams on study	5	5	5	5	5	5
Dams with implantations	3	5		5	4	5
Percent with implantations	60	100		100	80	100
Dams examined on Day 29	5	5	5	4	5	3
Dams with implantations	3	5	4	4	4	3
Percent with implantations	60	100	80	100	80	100
Corpora lutea						
Mean	10	13	17	15	14	14
S[a]	1.5	3.7	6.6	3.8	1.3	7.3
Implantations						
Mean	7	6	7	9	8	7
S	3.0	3.3	2.2	2.4	2.4	2.1
Implantation efficiency						
Mean	67.0	52.4	50.1	63.1	59.2	60.3
S	23.58	31.79	34.85	18.12	20.40	28.73
Litters with live fetuses[b]	3	5	4	4	4	3
Total live fetuses	19	31	28	34	32	26
Live fetuses						
Mean	6	6	7	9	8	9
S	3.5	3.3	2.2	2.5	2.8	0.6
Percent live fetuses						
Mean	100.0	100.0	100.0	100.0	100.0	100.0
S	0.00	0.00	0.00	0.00	0.00	0.00
Litters with dead fetuses	0	0	0	0	0	0
Litters with resorbed fetuses	2	0	0	0	1	0
Total resorbed fetuses	2	0	0	0	1	0
Resorbed fetuses						
Mean	1	0	0	0	0	0
S	0.6	0.0	0.0	0.0	0.50	0.0
Percent resorbed fetuses						
Mean	13.1	0.0	0.0	0.0	5.0	0.0
S	12.54	0.00	0.00	0.00	10.00	0.00

[a]S - Standard deviation.
[b]Includes data for live and resorbed fetuses obtained from females with implantations at scheduled cesarean section on Day 29 of gestation only; does not include data from females which died on test.

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006659

Reviewed By: Krystyna K. Locke, Toxicologist KKL 5/10/88 006658
Section II, Toxicology Branch (TS-769C)
Secondary Reviewer: Edwin R. Budd, Section Head
Section II, Toxicology Branch (TS-769C) 5/11/88

DATA EVALUATION REPORT

Study Type: Teratology - Rabbit

Doc. No.: 006658
TOX Chem No.: 363A
MRID No.: 144521
Project No.: 8-0126

Test Material: Avenge; Purity: 99.1%

Synonyms: Difenzoquat Methyl Sulfate; AC 84,777

Study Number(s): 6123-114; 981-84-102

Sponsor: American Cyanamid Company

Testing Facility: Hazelton Laboratories America, Inc., Madison, WI

Title of Report: A Teratology Study With AC 84,777 in Rabbits: Final Report

Author(s): Karen MacKenzie

Report Issued: December 14, 1984

Conclusions:

Teratogenic NOEL = > 100 mg/kg (mid-dose)*
Fetotoxic NOEL = > 100 mg/kg*
Maternal NOEL = 100 mg/kg
Maternal LEL = 250 mg/kg (HDT; high mortality and high percentage of animals with resorptions only)

Core Classification: Minimum

Experimental Procedures:

Artificially inseminated New Zealand rabbits (about 4 months old and weighing 3.5 kg), 18 animals/group, were tested with 0, 50, 100, or 250 mg AC 84,777/kg of body weight on gestation days (gd) 7 through 19. The compound was administered by gavage as a solution in distilled water. Immediately prior to insemination,

*Because of very high maternal mortality in the 250 mg/kg group (61%), insufficient pups (only 17) were available for a meaningful evaluation of the teratogenic potential of the test material at that level.

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each doe was injected with human chorionic gonadotropin solution (250 IU in 0.25 mL 0.9% saline). Cesarean sections were performed on gd 29 and all dams were examined macroscopically. Gross pathologic lesions were preserved in 10% phosphate-buffered formalin for possible future histopathologic examination. The reproductive tract was evaluated for the following:

- o Number and location of live and dead fetuses;
- o Early and late resorptions;
- o Empty sites and implantation scars;
- o Unusual coloration and variations in amniotic fluid or placentae; and
- o Any other abnormalities.

The ovaries were examined for gross abnormalities and the number of corpora lutea, and the gravid uterus was weighed. Uteri that appeared nongravid were opened and placed in a 10% solution of ammonium sulfide to confirm pregnancy status.

All viable fetuses were individually tagged for identification, weighed, examined externally for gross abnormalities, and then given a thorough examination of the viscera including internal sexing and a heart dissection; the brain was examined by a mid-coronal slice. All fetuses were then eviscerated, skinned, leared, stained, and evaluated for degree of skeletal ossification, variations, and malformations. Intact aborted or premature fetuses and fetuses obtained from dams not surviving until scheduled necropsy were examined externally and placed in 10% phosphate-buffered formalin.

The animals were:

- o Obtained from Hazelton Dutchland, Inc., Denver, PA;
- o Acclimated for at least 19 days;
- o Assigned randomly to test groups;
- o Housed individually;
- o Identified by numbered metal ear tags;
- o Fed unrestricted amounts of food (Purina Certified Rabbit Chow 5322, pelleted) and water;
- o Observed twice daily for toxic signs; and
- o Weighed on gd 0, 7, 12, 15, 18, 24, and 29.

Maternal and litter data were evaluated by appropriate statistical procedures (1, 2, 3, 4, and 5).

Results:

Maternal Data

Survival

Survival was very low in the high-dose group. Of the 18 inseminated females assigned to each group, 17 (94%), 15 (83%), 15

(83%), and 7 (39%) survived until the termination of the study (gd 29) in the control, low-, mid-, and high-dose groups, respectively. One female in each of the first three groups was sacrificed upon evidence of abortion (gd 20-26), and another female in the low-dose group was sacrificed because of a broken back. The remaining animals (1, 2, and 11 in the low-, mid-, and high-dose groups, respectively) died during gd 9 to 18.

Pregnancy Status

The overall pregnancy rates for the study were 94, 83, 89, and 83 percent for the control, low-, mid-, and high-dose groups, respectively. The pregnancy rates for the same groups, for animals surviving until the termination of the study, were 94, 80, 87, and 86 percent, respectively. At the termination of the study, a high percentage of animals with resorptions only, compared with the controls, was observed in the high-dose group. The incidence of these resorptions in the control, low-, mid-, and high-dose groups was 6, 17, 15, and 33 percent, respectively.

Toxic Signs

Possibly treatment-related observations included diarrhea in 7 out of 11 high-dose animals that died during the study, and single incidents of wetness around the muzzle and genital area in the same group.

Body Weights

No statistically significant differences were observed in mean body weights or weight changes, uncorrected and corrected for gravid uteri. On gd 29, the mean body weights (corrected for gravid uteri) were 3.25, 2.57, 3.27, and 3.45 kg for the control, low-, mid-, and high-dose groups, respectively.

Necropsy

There were no treatment-related findings. Ascites was observed in the control, low-, and mid-dose groups, but the incidence was higher in the controls. Fluid-filled lungs, noted in four animals (one in low-, one in mid-, and two in high-dose groups) that died during the study because of intubation errors were not attributed to treatment. Other observations noted at necropsy included a displaced kidney in one animal and a lobulated and fragmented spleen in another animal in the mid-dose group, and three fluid-filled cysts in the oviductal connective tissue of one animal in the high-dose group.

Litter Data

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The numbers of females examined at the cesarean section in the control, low-, mid-, and high-dose groups were 16, 12, 13, and 6, respectively.

There were no statistically significant differences in the mean number of corpora lutea or implants, implantation efficiency (number of implants x 100/number of corpora lutea), fetal body weights, sex ratio, or in the number of resorptions (total, early, and late). The mean number of live fetuses/dam in the treated groups was smaller than that of the control group but the difference was statistically significant ($p = < 0.05$) only for the low-dose group. Dead fetuses were not observed in this study. (For details, see Attachment I.)

The numbers of litters examined for visceral and skeletal abnormalities in the control, low-, mid-, and high-dose groups were 15, 10, 11, and 4, respectively. The numbers of fetuses examined in these groups were 119, 52, 70, and 17, respectively. There were no treatment-related visceral abnormalities. There was, however, a slight and possibly treatment-related increase in the number of fetuses with vertebral central abnormalities in the high-dose group. The fetal percent incidence of this skeletal malformation in the control, low-, mid-, and high-dose groups was 0, 0, 1, and 12, respectively. The litter percent incidence of the malformation in the same groups was 0, 0, 9, and 25, respectively. According to the submitted report, the increased incidence of vertebral central abnormalities was statistically significant in the high-dose group, but statistical significance was not designated in the data.

Maternal NOEL = 100 mg/kg
Maternal LEL = 250 mg/kg (HDT; high mortality and high percentage of animals with resorptions only)
Teratogenic NOEL = > 100 mg/kg*
Fetotoxic NOEL = > 100 mg/kg*

Core Classification: Minimum

A Quality Assurance Statement, dated December 14, 1984 was included in the report.

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*According to the author of this report, teratogenic (and presumably fetotoxic) NOEL was 250 mg/kg (HDT). TB/HED disagrees. Maternal mortality of 61 percent in this group resulted in insufficient numbers of pups (only 17) to permit meaningful evaluation of the teratogenic potential of the test material at the 250 mg/kg level.

REFERENCES

1. Sokal, R. R. and F. J. Rohlf, Biometry, W. H. Freeman and Company: San Francisco, Chapter 9, pp. 204-252 (1969).

2. Dunnett, C. W., "A Multiple Comparison Procedure for Comparing Several Treatments with a Control," J. Am. Stat. Assoc. 50, pp. 1096-1121 (1955).

3. Sokal, R. R. and F. J. Rohlf, Biometry, W. H. Freeman and Company: San Francisco, Chapter 13, pp. 367-403 (1969).

4. Dunn, O. J., "Multiple Comparisons Using Rank Sums," Technometrics 6, pp. 241-252 (1964).

5. Sokal, R. R. and F. J. Rohlf, Biometry, W. H. Freeman and Company: San Francisco, Chapter 16, pp. 549-620 (1969).

Attachment I

RIN 2218-94 TOX REVIEW FOR AVENGE (106401)

Page____ is not included in this copy.

Pages 89 through 93 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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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.

Reviewed by: Krystyna K. Locke, Toxicologist
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)

RRL 5/10/88
ERB 5/11/88

DATA EVALUATION REPORT

Study Type: Reproduction (3-gen.) -- Rat

Tox. Chem. No.: 363A
Doc. No.: 006658
MRID No.: 37924
Project No.: 3-0126

Test Material: AC 84,777;
Purity not specified.

Synonyms: Difenzoquat, Avenge

Study Number(s): 362-147

Sponsor: American Cyanamid Company

Testing Facility: Hazelton Laboratories, Inc.,
Vienna, VA

Title of Report: Final Report: Three-Generation Reproduction Study in Rats

Author(s): Reno, F. E.

Report Issued: October 14, 1974

Conclusions:

Parental NOEL = > 2500 ppm (125 mg/kg; HDT)*

Reproductive/Developmental NOEL = 500 ppm (25 mg/kg)

Reproductive/Developmental LEL = 2500 ppm (Decreased body weights of male and female pups at weaning in all generations; decreased body weights of male and female pups at birth in the second and third generation, respectively)

Core classification: Minimum

*Although, compared with the controls, there were statistically significant ($p < 0.05$) decreases in maternal body weights at this dose level during the premating period (the only time when body weights were recorded in this study), these were [illegible] small (5 or 6 %) and probably

biologically insignificant or occurred in animals which were smaller (20 %) than controls at the start of the treatment, due to reduced weight at weaning.

Experimental Procedures:

Charles River rats, 10 males and 20 females/ dose level, were fed diets containing 0, 500 or 2500 ppm of AC 84,777 for three successive generations. The feeding of the test material was started 14 weeks (P_1 parents) or 9 weeks (P_2 and P_3 parents) before mating and was continued throughout the mating, gestation and lactation periods. The test material was incorporated in Purina Laboratory Chow and the food (and water) were allowed in unrestricted amounts. The animals weighed 125-149 g (males) and 94-125 g (females) at the start of treatment, were assigned to groups randomly and housed individually. The parents were observed for toxic signs and mortality throughout the study. Individual body weights were recorded on treatment weeks 0, 4, and 9 (P_1, P_2 and P_3 animals) and also on treatment week 14 for the P_1 animals. Food intake was recorded during the same weeks, except that no record was made at the start of the treatment (week 0).

Twenty-four hours after parturition, the litters in each generation were randomly reduced to a maximum of eight pups (4 males and 4 females, when possible). A record was kept of the number of pregnancies, litters born, live and still births, deaths during lactation, and of litter size and weight by sex at 24 hours and at weaning. All pups were examined for external abnormalities. At the weaning of each generation, 10 males and 20 females from each dose level were saved for continuation of the study; about one-third of the pups were subjected to gross necropsy; and the remaining pups and all parents were discarded (without necropsy).

From 20 male and 20 female weanling pups of the third generation (F_{3A}), the following tissues were preserved in formalin for possible future histopathological examination:
brain, pituitary, eye, thyroid, lung, liver, spleen kidney, adrenal, stomach, pancreas, small intestine, urinary bladder, gonad, bone, bone marrow, heart, large intestine, and any unusual lesions.

The following reproductive indices were calculated for each generation: Fertility Index (number of pregnancies x 100 / number of females mated) and Gestation Index (number of litters born x 100 / number of pregnancies observed).

Statistical analysis of parental group mean body weight and food consumption data and of the reproduction data (mean number of pups born, mean number of live pups, mean number of dead pups, and mean weight of live pups at 24 hours and weaning) was

performed by the t-test at the 5.0% probability level[1].

Results:

Parental Data

Males -- The high-dose animals in all generations gained slightly less weight (4-7 %) than did the controls during the premating period, but these differences in weight gains were small and lacked statistical and, probably, biological significance. Also, compared with the controls, the P_2 and P_3 high-dose males weighed less (10-12 %) at the initiation of treatment, due to reduced weights at weaning.

Food consumption was occasionally reduced among the high-dose animals, but decreases were small (3-7 %), inconsistent, and, in most instances, statistically insignificant.

Females -- Compared with controls, decreases in body weights were observed in the high-dose animals (5 to 6 in P_1 and P_3 generations, and 11 to 15 % in the P_2 generation). These decreases generally persisted throughout the premating period (the only time when weight was recorded) and were statistically significant consistently for the P_1 and P_2 animals. Body weights among the low-dose animals were also occasionally slightly (6 to 8 %) but significantly ($p < 0.05$) lower than those of the controls. However, due to reduced weight at weaning, the P_2 animals weighed 20% less at the initiation of treatment than did the controls. This lower initial weight did not worsen as treatment with AC 84,777 was continued.

There were no consistent differences in food intake among the control and the treated groups in all generations.

Reproductive/Developmental Data

Male and female pups born in the high-dose group weighed less at weaning in each generation than did those in the control group. The mean weight decreases ranged from 13.9 to 15.5 % and were statistically significant. In the low-dose group, the mean weight decreases ranged from 2.1 to 8.7 % and all were statistically insignificant.

The test material had no effect on the fertility and gestation indices, number of pups born alive and dead, weight of live pups at birth (F_{1A} generation only) and pup mortality during the lactation period (F_{1A} and F_{3A} generations only).

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[1]Wilfred J. Dixon and Frank J. Massey, Jr., Introduction to Statistical Analysis, pp. 123-124, p. 232 - Section 13-6, McGraw-Hill, 1957.

Statistically significant decreases in the weight of the male pups (7 %) and female pups (8.2 %) at birth were observed in the F_{2A} and F_{3A} generations, respectively. These weight decreases were attributed to smaller size of the parents, compared with their respective controls. There was also an increase in the number of the high-dose male pups dying during the lactation period, but only int the F_{2A} generation. (For details on the above findings, see Attachment I).

Gross necropsy of randomly selected pups did not reveal treatment-related abnormalities. Incidental observations included a roughened spleen in one F_{1A} control female and one F_{3A} low-dose female; thickened stomach walls of one F_{3A} female (dose level not specified); and thinness of two F_{3A} high-dose males.

Comments:

This study was conducted some 14 years ago and some of the data required in the guidelines are missing, such as: 1) Parents were not necropsied (and neither was one low-dose female which was found dead during the premating period); 2) Animals were weighed only during the premating period, a total of four times during 14 weeks (P_1 group) and a total of three times during 9 weeks (P_2 and P_3 groups); and 3) Litters were weighed only at birth and at weaning. However, enough of the essential data were reported to accept this study as an adequate reproduction study. Although two levels, rather than at least three, of the test material were used, the highest level caused developmental effects.

Parental NOEL = > 2500 ppm (125 mg/kg; HDT. See page 1 for comments)

Reproductive/Developmental NOEL = 500 ppm (25 mg/kg)

Reproductive/Developmental LEL = 2500 ppm (See page 1 for toxic signs)

Core Classification: Minimum

Quality Assurance Statement was not included in the report.

Attachment I

RIN 2218-94 TOX REVIEW FOR AVENGE (106401)

Page_____ is not included in this copy.

Pages 99 through 101 are not included.

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____ Identity of product inert ingredients.

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006658

Reviewed by: Krystyna K. Locke, Toxicologist KKL 5/20/88
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Edwin R. Budd, Section Head
Section II, Tox. Branch (TS-769C)
Secondary reviewer: Kerry L. Dearfield, Ph.D.
Scientific Mission Support Staff, Tox. Branch (TS-769C) 5-20-88

DATA EVALUATION REPORT

Study Type: Mutagenic (Dominant lethal)--rat

Tox. Chem. No.: 363A
MRID No.: 30577
Project No.: 8-0126
Doc. No.: 006658

Test Material: Technical AC 84,777; white crystalline material; Lot No. AC-1786-158; purity not stated

Synonyms: Difenzoquat; Avenge

Study Number(s): 2088

Sponsor: American Cyanamid Co.

Testing Facility: Food and Drug Research Laboratories, Inc.

Title of Report: Dominant Lethal Study in Rats with AC 84,777

Author(s): Bailey, D. E. and Morgareidge, K.

Report Issued: September 3, 1974

Conclusions:

Classification: Unacceptable. This classification can be upgraded to acceptable upon receipt of appropriate positive control data.

The test material was not mutagenic in this study, as judged by the incidence of embryonic mortality, when the females mated with treated males were compared with those mated with untreated males. Males were fed unrestricted amounts of food containing 0, 500 or 2500 ppm of technical AC 84,777 for 60 days before they were mated (1:1) with untreated females. Mating schedule was repeated for 8 consecutive weeks. Cesarean sections were performed on gestation day 13. Positive control was not used. No rationale was given for the selection of dose levels, but these levels were also used by the same testing facility in a 2-year rat feeding study. Since decreased body weight gains were observed in both studies at the 2500 ppm level, this level appears adequate as a high-dose level. Statistical analyses were not used in the dominant lethal study.

This study was originally evaluated by Toxicology Branch in 1974 (see Attachment I). That very brief review, which is factually correct, is currently amended as follows:

1. Body weights and food consumption of the males were recorded weekly throughout the study and efficiency of food utilization (EFU; grams gained/100 g of food eaten) was also calculated. Compared with the controls, the low-dose and high-dose males gained 10.2 and 13.2 percent less weight, respectively.

2. The mutagenic effects (incidence of embryonic mortality) was determined in terms of the numbers of corpora lutea (C.L.), implantation sites (I.S.), live fetuses (L.F.) and resorption sites (R.S.). The following indices were also calculated: I.S. x 100/C.L., L.F. x 100/I.S. and R.S. x 100/I.S. (Mutagenic Index). Data were reported for individual animals and in terms of group means.

3. Strain and source of animals, procedures used in assigning animals to groups, type of diet fed, and housing conditions were not reported.

4. The Mutagenic Index for the control, low-dose and high-dose groups was 3.83, 3.72 and 4.23 percent, respectively, indicating that the test material was not mutagenic in this study. A slight increase in the Mutagenic Index for the high-dose does not appear to be biologically significant.

5. While this study was apparently conducted in 1974, there appears to be enough information to consider dominant lethal effects as compared to a negative control. However, a concurrent positive control was not reported. Therefore, the sensitivity of the system (unknown rat strain) and proficiency of the laboratory at the test time have not been demonstrated. Historical positive control data from the time period of this test (within 1 year time frame) would be acceptable (with the same rat strain). The classification of unacceptable can be upgraded to acceptable upon receipt of appropriate, adequate positive control data.

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Attachment I

Dominant Lethal Study in Rats:

0, 500, and 2500 ppm were administered to 3 groups of 15 male weanling rats, respectively, for 60 days. These males were then mated 1:1 with untreated virgin females, and caesarean sections performed on day 13 of gestation. Mating schedule was repeated with virgin female rats bred 1:1 with the treated or control males for 8 consecutive weeks. Results of this study demonstrated no mutagenic effect for either of the test groups.