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

010689

DEC 3 , 1993

OFFICE OF
PREVENTION, PESTICIDES &
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: BARDAC 2180: Review toxicology data to support Registrant's request for registration of a new chemical

EPA IDENTIFICATION NUMBERS:

P.C. Code: 069207
Caswell No.: New chemical
DP Barcodes: D188673, D189620
Submission No.: S436.

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke, 1 Dec 91
Toxicology Branch II, Section IV
Health Effects Division (H7509C)

TO: John Lee
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THRU: Jess Rowland, M.S. Jess Rowland 12/01/93
Toxicology Branch II, Head, Section IV
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and

Marcia van Gemert, Ph.D. Marcia van Gemert 12/1/93
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Health Effects Division (H7509C)

Registrant: Lonza, Inc., Fair Lawn, NJ

Chemicals: BARDAC 2180, ISOBARDAC
Decanaminium, N-isononyl-N,N-diethyl, chloride

Action Requested: Review studies to support registration BARDAC 2180 as a new chemical for an aquatic, general use pattern. Proposed uses of BARDAC 2180 are as a algicide for treating recirculating cooling towers, swimming pools and spas.

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1. Background: The data package submitted for review consists of acute toxicity studies for BARDAC 2180 (ISOBARDAC) and various subchronic and chronic studies for two other quaternary ammonium salts (QUATs), ADBAC (N-alkyl dibenzyl ammonium chloride, PC Code: 069105, Caswell No.: 016E) and BARDAC 2280 (80% ai)/2250 (50% ai) (didecyldimethylammoniumchloride, PC Code: 069149, Caswell No.: 331A).

Rather than requiring separate data packages for each individual QUAT, the Agency adopted a clustering approach (PR Notice 88-2, dated 26 February 1988), which permitted representative members of each cluster to be used in toxicity studies, instead of requiring separate studies on each QUAT.

BARDAC 2180 and BARDAC 2280/2250 are both Group I QUATs. Therefore, the acute studies with BARDAC 2180 and the subchronic, chronic, developmental and reproductive toxicity studies for BARDAC 2280/2250 can be used to support the registration of BARDAC 2180. However, the studies pertaining to ADBAC (a Group 2 QUAT) cannot be used be used to support the registration of BARDAC 2180. The ADBAC toxicity studies were, however, reviewed.

2. Summary of Acute Toxicity Studies for BARDAC 2180

a. Acute Oral Toxicity in Rats - Median Lethal Dosage Determination with ISOBARDAC (MRID No.: 424770-05)

Male and Female LD_{50}: 224 mg/kg (a.i.)

Toxicity category II

CORE CLASSIFICATION - Guideline

b. Primary Skin Irritation Study in Rabbits with ISOBARDAC (MRID No.: 424770-07)

Toxicity category I (Corrosive)

CORE CLASSIFICATION: Guideline

c. Photoallergy Study in Guinea Pigs with ISOBARDAC (MRID No.: 424770-06)

CORE CLASSIFICATION: Unacceptable (Supplemental data, no guideline requirements)

3. Summary of Toxicity Studies for BARDAC 2280/2250:

a. BARDAC 2250: 90-day feeding study in dogs with a quaternary ammonium sanitizer Bardac-22 (MRID No.: 402629-01)

RESULTS: In a 90-day feeding study, male and female beagle dogs were given BARDAC 2250 at dosages of 0, 5, 15 or 50

mg/kg body weight/day. All animals survived to terminal sacrifice. Individual clinical signs were not recorded during the study. High-dose males and females experienced marked decrease in body weight gain, food consumption and food efficiency. Clinical chemistry, hematology, urinalysis, and pathology results did not reveal any treatment-related effects. The LOEL is based on decreased body weight gain, food consumption and food efficiency.

NOEL	LOEL
15 mg/kg/day	50 mg/kg/day

CORE CLASSIFICATION: This study is classified as Supplementary since individual clinical signs and analytical data were not provided. This study is acceptable for regulatory purposes and satisfies the guideline requirement [§82-1(a)], since a 1-year oral study in this species (MRID No. 419704-01) is available.

b. BARDAC 2280: Ninety-day subchronic oral toxicity study with didecyldimethylammoniumchloride (MRID No.: 409663-02)

RESULTS: For 13 weeks, male and female rats were given diets containing 0, 100, 300, 600, 1000, or 3000 ppm (respective mg/kg/day equivalents: 0, 6.2, 18.5, 36.8, 60.7 and 175.4 for males; 0, 7.5, 22.3, 44.4, 74.3 and 225.5 for females) of BARDAC 2280. LOEL is based on increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidences of gross pathological observations and non-neoplastic lesions. From the results of this study, NOEL and LOEL are:

	NOEL	LOEL
Males	1000 ppm (60.7 mg/kg/day)	3000 ppm (175 mg/kg/day)
Female	1000 ppm (74.3 mg/kg/day)	3000 ppm (225 mg/kg/day)

CORE CLASSIFICATION: Guideline

c. BARDAC 2230: Ninety-day subchronic dermal toxicity study with didecyldimethylammoniumchloride in rats (MRID No.: 413059-01)

RESULTS: Male and female Sprague-Dawley rats received repeated dermal dosing of the BARDAC 2280 at 0, 2, 6, or 12 mg/kg/day for 6 hours/day, 5 days/week for 13 weeks. No treatment-related effects were noted in mortality, weight gain, food consumption, or systemic toxicity. Toxicity was limited to treated skin of mid-dose females and high-dose males and females. Gross dermal lesions (erythema, edema, exfoliation, excoriation and ulceration) were confirmed by histopathological examination, where increased incidence of hyperkeratosis, acanthosis, epidermitis, dermatitis and

ulceration were noted.

From the results of this study, NOELs and LOELs for systemic and dermal toxicity are as follows:

Toxicity	NOEL	LOEL
Systemic	> 12 mg/kg/day (HDT)	
Dermal Males	6 mg/kg/day	12 mg/kg/day
Females	2 mg/kg/day	6 mg/kg/day

The dermal LOEL is based is based increased incidence of histopathological lesions (hyperkeratosis, males and females and acanthosis, epidermitis, dermatitis and ulceration in females).

CORE CLASSIFICATION: Minimum. This study is acceptable for regulatory purposes and satisfies the guideline requirement [§82-2], since a 90-day oral study (MRID No.: 409663-02) establishing syscemic toxicity in this species is available.

d. BARDAC 2280: Chronic oral toxicity study of didecyl-dimethylammoniumchloride in dogs (MRID No.: 419704-01)

RESULTS: In a chronic, 1-year oral toxicity study, male and female beagle dogs were given BARDAC 2280 at dosages of 0, 3, 10, or 20/30 mg/kg body weight/day (Dosing at 30 mg/kg/day was not tolerated well and was discontinued on Day 31; dosing was resumed on Day 36 at 20 mg/kg/day). No treatment-related deaths occurred during the study. The treatment-related clinical signs (soft/mucoid feces, emesis) were observed frequently in high-dose animals. Hematology or urinalysis results were normal. Total cholesterol levels were significantly decreased in the high-dose females. Gross and histopathological findings did not reveal any treatment-related effects.

	NOEL	LOEL
Males & females	10 mg/kg/day	20 mg/kg/day (HDT)

The LOEL is based on increased incidence of clinical observations (emesis and soft/mucoid feces) in males and females and decreased total cholesterol levels in females.

CORE CLASSIFICATION: Guideline

e. BARDAC 2280: Chronic dietary oncogenicity study with didecyldimethylammoniumchloride in mice (MRID NO.: 418923-01)

RESULTS: Male and female mice were fed diets containing 0, 100, 500 or 1000 ppm BARDAC 2280 (mg/kg/day equivalents: 0, 15.0, 76.3, or 155.5 for males and 18.6, 93.1, 193.1 for females). No treatment-related effects were noted in the

incidence of clinical signs, deaths, gross and histopathological observations, or incidence of adenomas and carcinomas. Hematological values were comparable among all study groups. Effects attributable to treatment at 1000 ppm (LOEL) included decreased mean body weights and body weight gains of high-dose males and females.

	NOEL	LOEL
Males	500 ppm (76.3 mg/kg/day)	1000 ppm (155 mg/kg/day)
Females	500 ppm (93.1 mg/kg/day)	1000 ppm (193 mg/kg/day)

CORE CLASSIFICATION: Guideline

f. BARDAC 2280: Chronic dietary toxicity/oncogenicity study with decyldimethylammoniumchloride (MRID No.: 419651-01)

RESULTS: Male and female rats were fed diets containing BARDAC 2280 at 0, 300, 750 or 1500 ppm (mg/kg/day equivalents: 0, 13, 32, or 64 for males and 0, 16, 41, or 83 for females) for two years. High-dose animals showed significant, but slight (< 10%) decreases in mean body weight during the study. Treatment related effects consisted of increased incidence of sinusoidal blood, hemosiderosis and histiocytosis in the mesenteric lymph nodes of high dose animals. The incidence of neoplastic lesions in treated animals was comparable to controls. BARDAC 2280 was not carcinogenic in male or female rats.

	NOEL	LOEL
Males	750 ppm (32 mg/kg/day)	1500 ppm (64 mg/kg/day)
Females	750 ppm (41 mg/kg/day)	1500 ppm (83 mg/kg/day)

The LOEL is based on increased incidence on nonneoplastic lesions in the mesenteric lymph nodes (sinusoidal blood, hemosiderosis and histiocytosis).

CORE CLASSIFICATION: Minimum

g. BARDAC 2280: Developmental toxicity evaluation of didecyldimethyl-ammoniumchloride administered by gavage to CD (Sprague-Dawley) rats (MRID No.: 418867-01)

RESULTS: In a developmental toxicity study, Sprague-Dawley rats were administered BARDAC 2280 daily by gavage at dose levels of 0, 1, 10 or 20 mg/kg/day on gestation days 6-15, inclusively. Maternal toxicity at 10 mg/kg/day included clinical signs (audible respiration). At 20 mg/kg/day, maternal animals exhibited clinical signs (audible

respiration, gasping), decreased body weight gain and decreased food consumption during dosing period. No developmental toxicity was noted at the highest dose tested.

	NOEL	LOEL
Maternal	1 mg/kg/day	10 mg/kg/day
Developmental	20 mg/kg/day	not determined

CORE CLASSIFICATION: Guideline

h. BARDAC 2280: Developmental toxicity study of didecyldimethylammonium-chloride administered by gavage to New Zealand white rabbits (MRID No.: 410187-01)

RESULTS: In a developmental toxicity study, NZ white rabbits were administered BARDAC 2280 daily by gavage at dose levels of 0, 1, 3, or 10 mg/kg/day on gestation days 6-18, inclusively. At 3 and 10 mg/kg/day maternal animals exhibited clinical signs (hypoactivity, labored and/or audible respiration) and decreased body weight gain during dosing period. At 10 mg/kg/day there was increased incidence of mortality. Developmental toxicity included decreased fetal body weight and increased number of dead fetuses at 10 mg/kg/day.

	NOEL	LOEL
Maternal	1 mg/kg/day	10 mg/kg/day
Developmental	3 mg/kg/day	10 mg/kg/day

CORE CLASSIFICATION: Minimum

i. BARDAC 2280: Two-generation reproduction study in Sprague-Dawley (CD) rats with didecyldimethylammonium-chloride administered in diet (MRID No.: 418045-01)

RESULTS: In a two-generation reproduction study, Sprague-Dawley (CD) rats were fed BARDAC 2280 at dietary levels of 0, 300, 750 or 1500 ppm (premating: 0, 20, 50, 103 mg/kg/day, males; 0, 24, 61, or 122 mg/kg/day, females). Parental toxicity at 1500 ppm consisted of decreased body weight/body weight gain. Reproductive toxicity at 1500 ppm consisted of decreased mean pup body weight/body weight gain during the postnatal period.

	NOEL	LOEL
Parental, premating	750 ppm	1500 ppm
Males:	(50 mg/kg/day)	(103 mg/kg/day)
Females:	(61 mg/kg/day)	(122 mg/kg/day)
Reproductive	750 ppm	1500 ppm

CORE CLASSIFICATION: Guideline

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j. BARDAC 2280: Analysis of metaphase chromosomes obtained from bone marrow of rats treated with P0151 (MRID No.: 407058-02)

RESULTS: Negative

CORE CLASSIFICATION: Unacceptable

k. BARDAC 2280: Chromosomal aberrations assay with chinese hamster ovary cells in vitro (MRID No.: 412526-01)

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

l. BARDAC 2280: Mutagenicity test on didecyldimethyl-ammoniumchloride (DDAC) in the CHO/HGPRT forward mutation assay (MRID No.: 408952-02)

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

m. BARDAC 2280: Mutagenicity test on didecyldimethyl-ammoniumchloride in the rat primary hepatocyte assay (MRID No.: 408952-01)

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

n. BARDAC 22: Absorption, distribution, metabolism and excretion studies of didecyldimethylammoniumchloride (DDAC) in the rat (MRID No.: 416171-01, main study) and Absorption, distribution, metabolism and excretion studies of didecyl-dimethylammoniumchloride (BARDAC 22) in the rat (MRID No.: 413851-01, addendum).

RESULTS: The absorption, distribution, metabolism and excretion of ^{14}C-BARDAC 22 was studied in male and female rats. For the single dose experiments, rats were orally gavaged with 10 mg/kg or 50 mg/kg; for the repeated low dose study animals were fed a diet containing unlabeled BARDAC 22 at 100 ppm in diet for 14 days, followed by single oral gavage dose of ^{14}C-BARDAC at 10 mg/kg of Day 15. Total recovery ranged from 90.8 to 100.9% of the administered dose. Fecal elimination predominated and accounted for 89.11 to 99.46% of the dose. Bioaccumulation of BARDAC 22 was low (< 1%). The proposed metabolic pathway for BARDAC 22 suggests that oxidization occurs at the two decyl side chains (probably at or near the terminal end) to form hydroxy and hydroxyketo derivatives. NS data indicated that 2-methyl substituents of BARDAC 22 remained unchanged. Less unchanged parent compound was present in the feces of

females, suggesting a greater amount of metabolism. The extent to which biliary excretion and/or retention of parent compound in the gut contributed to the high fecal elimination could not be evaluated because an i.v. study was not performed.

CORE CLASSIFICATION: Supplementary

4. Summary of Toxicity Studies for ADBAC:

a. Ninety-day dietary toxicity study with alkyl dimethyl benzyl ammonium chloride (ADBAC) (MRID No.: 407466-01)

RESULTS: For 13 weeks, male and female rats were given diets containing 0, 100, 500, 1000, 4000, or 8000 ppm of ADBAC. The equivalent doses in mg/kg/day for the 100, 500, 1000, 4000 (estimated) and 8000 (estimated) ppm groups were 6.3, 31.2, 62.0, ≈ 248 and ≈ 496 for males and 0, 7.9, 38.1, 76.7, ≈ 308 and ≈ 616 for females. Treatment-related mortality was limited to the 4000 ppm (80% males and 73% females) and 8000 ppm (100% for males and females) groups. The 4000 ppm animals also showed decreased mean body weights, body weight gain and food consumption and increased incidence of gross and microscopic lesions.

	NOEL	LOEL
Males	500 ppm (31.2 mg/kg/day)	1000 ppm 62.0 mg/kg/day)
Females	1000 ppm (76.7 mg/kg/day)	4000 ppm (308 mg/kg/day)

The LOEL is based on decreased body weight and body weight gain in males and increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidence of gross and microscopic lesions in females.

CORE CLASSIFICATION: Guideline

b. Teratologic evaluation of four quaternary compounds (Barquat MB-50; Barquat MX-50; Barquat 4250; Barquat 4250Z (an unpublished review of teratology studies submitted by Waverly Research Center, Food and Drug Research Laboratories, Inc) (MRID No.: 228149)

Four quaternary compounds were evaluated for potential of inducing developmental toxicity in Wistar albino rats following oral administration at 0, 10, 25, or 50 mg/kg/day during gestational days (GD) 6-15, inclusively. Based on the summary data, neither maternal nor developmental toxicity was observed in these studies. A lack of maternal toxicity at the highest dose level indicates an improper selection of that dose level. However, these conclusions cannot be verified since a lack of individual data and

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rationale for the selection of dose levels were not provided. In addition, compliance statements and analytical chemistry data were not submitted.

CORE CLASSIFICATION: Supplementary

c. Series 84-2 assessment of the mutagenic activity of ityamine 3500 in the mouse nucleus test (MRID No.: 403111-01)

RESULTS: Negative

CORE CLASSIFICATION: Unacceptable

d. Genotoxicity test on alkyl dimethyl benzyl ammonium chloride (ADBAC) in the assay for unscheduled DNA synthesis in rat liver primary cell cultures (MRID No.: 422908-01)

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

e. Mutagenicity test on alkyl dimethyl benzyl ammonium chloride (ADBAC) in the CHO/HGPRT forward gene mutation assay (MRID No.: 410127-01

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

f. Absorption, distribution, metabolism and excretion of alkyl dimethyl benzyl ammonium chloride (ADBAC) in the rat (MRID No.: 409907-01)

RESULTS: The pharmacokinetic profile of ^{14}C-ADBAC was studied in male and female rats orally gavaged with low (10 mg/kg), high (50 mg/kg) or repeated low dose (100 ppm in diet for 14 days followed by single dose at 10 mg/kg) of test compound, additionally an i.v. low (10 mg/kg) dose study was also performed. Fecal elimination accounted for > 90%, while 5.8 to 7.0% appeared in the urine. For i.v. dosing 20 to 30% appeared in the urine with 44 to 55% in the feces. Tissue accumulation of orally-dosed animals negligible (< 1% of administered dose), while the carcasses and tissues of i.v. dosed animals retained 33.4% (males) and 35.8% (females) of the administered dose. Identification of metabolites were not performed in this study.

CORE CLASSIFICATION: Supplementary. The study may be upgraded to guideline if metabolic profile data are submitted and judged to be acceptable.

010699

5. Summary of Toxicology Database for BARDAC 2180 and BARDAC 22:

Guideline		Required/Satisfied	MRID No.
Acute Toxicity Studies with BARDAC 2180			
§81-1	Acute Oral - Rat	Yes/Yes	424770-05
§81-2	Acute Dermal	No[a]	
§81-3	Acute Inhalation - Rat	No[b]	
§81-4	Primary Eye Irritation - Rabbit	No[a]	
§81-5	Primary Dermal Irritation	Yes/Yes	424770-07
§81-6	Primary Dermal Photoallergy	No/No	424770-06
Subchronic Toxicity Studies with BARDAC 2280/2250			
§82-1(a)	90-Day Oral - Rat	Yes/Yes	409663-02
§82-1(b)	90-Day Oral - Dog	Yes/Yes	402629-01
§82-3	90-Day Dermal - Rat	Yes/Yes	413059-01[e]
Chronic Toxicity Studies with BARDAC 2280			
§83-1(b)	Chronic Oral Toxicity - Dog	Yes/Yes	419704-01
§83-2(b)	Carcinogenicity - Mouse	Yes/Yes	418023-01
§83-5(a)	Chronic Oral/Carcinogenicity - Rat	Yes/Yes	419651-01
Reproductive Toxicity Studies with BARDAC 2280			
§83-3(a)	Developmental Toxicity - Rat	Yes/Yes	418867-01
§83-3(b)	Developmental Toxicity - Rabbit	Yes/Yes	418187-01
§83-4	Reproduction, 2-Generation	Yes/Yes	418045-01
Mutagenicity Studies with BARDAC 2280/2250			
§84-2	Gene Mutation	Yes/Yes	408952-02
	Structural Chrom. Aberrations	Yes/Yes Yes/No	412526-01[c] 407058-02
§84-4	Other Genotoxic Effects	Yes/Yes	408952-01[c]
Metabolism			
§85-1	Metabolism, general	Yes/No	416171-01[d] 413851-01

[a] Material corrosive
[b] Material not inhalable under conditions of use
[c] Original review of study graded as unacceptable, upgraded to acceptable on rereview
[d] Original review of study graded as acceptable, changed to unacceptable on rereview

6. Recommendations: The current toxicology database for BARDAC 2180 and BARDAC 2280/2250 is adequate to support registration of BARDAC 2180 as a new chemical.

7. Data Gaps: Two data gaps were identified: (1) General metabolism (§85-1) was graded as supplementary because an i.v. study is requested and (2) Mutagenicity: In vivo cytogenetic assay with rats (§84-2) was graded as unacceptable because the magnitude of the positive control response was not great enough.

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NEW INPUT

010689

One-liner

CITATION

STUDY TYPE: Acute Oral - Rat (§81-1)

STUDY: Acute Oral Toxicity in Rats - Median Lethal Dosage Determination with ISOBARDAC

LABORATORY: Hill Top Biolabs, Inc., Miamiville, OH 45147

REPORT NO./DATE: 91-8357-21(A)/5 March 1992

EPA ACCESSION NO.: 424770-05

TEST MATERIAL: ISOBARDAC

BARDAC 2180

1-Decanaminium, N-isononyl-N,N-dimethyl-, chloride

P.C. CODE: 069207

CASWELL NO.: new chemical

RESULTS: Animals were orally gavaged with test compound as doses of 50, 130, 200, 320 or 500 mg/kg body weight (doses not corrected for percent active ingredient).

Male and Female LD_{50}: 224 mg/kg (corrected for percent a.i.)
275 mg/kg (not corrected for a.i.)

Toxicity category II

CORE CLASSIFICATION - Guideline

This study satisfies guideline requirement (81-1) for an acute oral toxicity study in the rat and is acceptable for regulatory purposes.

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NEW INPUT

One-liner

CITATION

STUDY TYPE: Primary Skin Irritation in Rabbits (§81-5)

STUDY: Primary Skin Irritation Study in Rabbits with ISOBARDAC

LABORATORY: Hill Top Biolabs, Inc., Miamiville, OH 45147

REPORT NO./DATE: 91-8357-21(C)/26 February 1992

EPA ACCESSION NO.: 424770-07

TEST MATERIAL: ISOBARDAC

BARDAC 2180

1-Decanaminium, N-isononyl-N,N-dimethyl-, chloride

P.C. CODE: 069207

CASWELL NO.: new chemical

RESULTS: Following a single application of test compound (0.5 ml) to the back of a single NZ white rabbit, erythema and edema developed early (30 min to 1 hr) and produced moderate to severe erythema (with necrosis) and severe edema after 24 hours.

TOXICITY CATEGORY I (Corrosive)

CORE CLASSIFICATION - Guideline

This study satisfies guideline requirement (§81-5) for a primary skin irritation study in rabbits and is acceptable for regulatory purposes.

NEW INPUT

010 E59

One-liner

CITATION

STUDY TYPE: Photoallergy study - Guinea Pigs

STUDY TITLE: Photoallergy Study in Guinea Pigs with ISOBARDAC

LABORATORY: Hill Top Biolabs, Inc., Miamiville, OH 45147

REPORT NO./DATE: 91-8357-21 (E)/26 February 1992

EPA ACCESSION NO.: 424770-06

TEST MATERIAL: ISOBARDAC

BARDAC 2180

1-Decanaminium, N-isononyl-N,N-dimethyl-, chloride

P.C. CODE: 069207

CASWELL NO.: new chemical

RESULTS: The study concluded that the test compound was not a photoallergen, however, the lack of a significant positive response indicates that the assay was not sensitive enough to detect weak photoallergens.

CORE CLASSIFICATION: Unacceptable (Supplemental data, no guideline requirements)

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NEW ITEM

One-liner

CITATION

STUDY: 90-day feeding study in dogs with a quaternary ammonium sanitizer Bardac-22

LABORATORY: Food and Drug Research Laboratories, Inc.

REPORT NO./DATE: 2224a/7 April 1975

EPA ACCESSION NO.: 402629-01

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 50% [ai]

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: In a 90-day feeding study, male and female beagle dogs were given test compound at dosages of 0, 5, 15 or 50 mg/kg body weight/day. All animals survived to terminal sacrifice. Individual clinical signs were not recorded during the study. High-dose males and females experienced marked decrease in body weight gain, food consumption and food efficiency. Clinical chemistry, hematology, urinalysis, and pathology results did not reveal and treatment-related effects.

	NOEL	LOEL
Males and females	15 mg/kg/day	50 mg/kg/day

The LOEL is based on decreased body weight gain, food consumption and food efficiency.

CORE CLASSIFICATION: This study is classified as Supplementary since individual clinical signs and analytical data were not provided. This study is acceptable for regulatory purposes and satisfies the guideline requirement [§82-1(a)], since a 1-year oral study in this species (MRID No. 419704-01) is available.

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NEW INPUT

010689

One-liner

CITATION

STUDY: Ninety-day subchronic oral toxicity study with didecyldimethylammoniumchloride

LABORATORY: Bushy Run Research Center

REPORT NO./DATE: 51-506/6 September 1988

EPA ACCESSION NO.: 409663-02

TEST MATERIAL: BARDAC 2280

Didecyldimethylammoniumchloride

Purity: 80.8%

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: For 13 weeks, male and female rats were given diets containing 0, 100, 300, 600, 1000, or 3000 ppm (respective mg/kg/day equivalents: 0, 6.2, 18.5, 36.8, 60.7 and 175.4 for males; 0, 7.5, 22.3, 44.4, 74.3 and 225.5 for females). LOEL is based on increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidences of gross pathological observations and non-neoplastic lesions. From the results of this study, NOEL and LOEL are:

	NOEL	LOEL
Males	1000 ppm (60.7 mg/kg/day)	3000 ppm (175.4 mg/kg/day)
Females	1000 ppm (74.3 mg/kg/day)	3000 ppm (225.5 mg/kg/day)

CORM CLASSIFICATION: Guideline

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UPDATE

One-liner

CITATION

STUDY: Ninety-day subchronic dermal toxicity study with didecyldimethylammoniumchloride in rats (§82-3)

LABORATORY: Bushy Run Research Center

REPORT NO./DATE: 51-554/October 7, 1988

EPA ACCESSION NO.: 413059-01

TEST MATERIAL: BARDAC 2280

Didecyldimethylammoniumchloride

Purity: 80.8%

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: Male and female Sprague-Dawley rats received repeated dermal dosing of the test compound at 0, 2, 6, or 12 mg/kg/day for 6 hours/day, 5 days/week for 13 weeks. No treatment-related effects were noted in mortality, weight gain, food consumption, or systemic toxicity. Toxicity was limited to treated skin of mid-dose females and high-dose males and females. Gross dermal lesions (erythema, edema, exfoliation, excoriation and ulceration) were confirmed by histopathological examination, where increased incidence of hyperkeratosis, acanthosis, epidermitis, dermatitis and ulceration were noted.

From the results of this study, NOELs and LOELs for systemic and dermal toxicity are as follows:

Toxicity		NOEL	LOEL
Systemic		> 12 mg/kg/day (HDT)	
Dermal	Males	6 mg/kg/day	12 mg/kg/day
	Females	2 mg/kg/day	6 mg/kg/day

The dermal LOEL is based is based increased incidence of histopathological lesions (hyperkeratosis, males and females and acanthosis, epidermitis, dermatitis and ulceration in females).

CORE CLASSIFICATION: Minimum. This study satisfies guideline requirements (§82-3) for a 90-day dermal toxicity study in rats and is acceptable for regulatory purposes. Although systemic toxicity was not noted in this study, a systemic LOEL was established in a 90-day oral study in rats (MRID No.: 409663-02).

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NEW ITEM

010689

One-liner

CITATION

STUDY TYPE: Chronic oral - dogs [§83-1(b)]

STUDY TITLE: Chronic oral toxicity study of didecyldimethyl-ammoniumchloride in dogs

LABORATORY: Hazleton Washington, Inc., Vienna, VA

REPORT NO./DATE: 2545-102/26 July 1991

EPA ACCESSION NO.: 419704-01

TEST MATERIAL: BARDAC

Didecyldimethylammoniumchloride

Purity: 80.8% [ai]

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: In a chronic, 1-year oral toxicity study, male and female beagle dogs were given test compound at dosages of 0, 3, 10, or 20/30 mg/kg body weight/day (Dosing at 30 mg/kg/day was not tolerated well and was discontinued on Day 31; dosing was resumed on Day 36 at 20 mg/kg/day). No treatment-related deaths occurred during the study. The treatment-related clinical signs (soft/mucoid feces, emesis) were observed frequently in high-dose animals. Hematology or urinalysis results were normal. Total cholesterol levels were significantly decreased in the high-dose females. Gross and histopathological findings did not reveal any treatment-related effects.

	NOEL	LOEL
Males and females	10 mg/kg/day	20 mg/kg/day (HDT)

The LOEL is based on increased incidence of clinical observations (emesis and soft/mucoid feces) in males and females and decreased total cholesterol levels in females.

CORE CLASSIFICATION: Guideline

NEW ITEM

One-liner

CITATION

STUDY TYPE: Carcinogenicity - mice [§83-2(b)]

STUDY TITLE: Chronic dietary oncogenicity study with didecyldimethylammoniumchloride in mice

LABORATORY: Hazleton Washington, Inc., Vienna, VA

REPORT NO.(DATED): 53-528 (7 February 1991)

EPA ACCESSION NO.: 418023-01

TEST MATERIAL: BARDAC 2280

Didecyldimethylammoniumchloride

Purity: 80.8% [a.i.]

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: In this carcinogenicity study, male and female mice were f diets containing BARDAC 2280 at concentrations of 0, 100, 500, or 1(ppm (mg/kg/day equivalents: 0, 15.0, 76.3, or 155.5 for males and (18.6, 93.1, or 193.1 for females). No treatment-related effects wer noted in the incidence of clinical signs, deaths, gross and histopathological observations. Hematological values were comparabl among all study groups. Effects attributable to treatment included decreased mean body weights and body weight gains of high-dose male and females. BARDAC 2280 was not carcinogenic in male or female mic

	NOEL	LOEL
Males and females	500 ppm (76.3 mg/kg/day)	1000 ppm (155.5 mg/kg/day)

The LOEL is based on decreased mean body weights and body weight gains.

CORE CLASSIFICATION: Minimum

19

NEW ITEM

010689

One-liner

CITATION

STUDY TYPE: Combined Chronic/oncogenicity - Rats [83-5(a)]

STUDY TITLE: Chronic dietary toxicity/oncogenicity study with decyldimethylammoniumchloride

LABORATORY: Bushy Run Research Center, Export, PA

REPORT NO./DATE: 53-566/27 June 1991

EPA ACCESSION NO.: 419651-01

TEST MATERIAL: BARDAC 2280

Didecyldimethylammoniumchloride

Purity: 80.8% [a.i.]

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: Male and female rats were fed diets containing BARDAC 2280 at 0, 300, 750 or 1500 ppm (mg/kg/day equivalents: 0, 13, 32, or 64 for males and 0, 16, 41, or 83 for females) for two years. High-dose animals showed significant, but slight (< 10%) decreases in mean body weight during the study. Treatment related effects consisted of increased incidence of sinusoidal blood, hemosiderosis and histiocytosis in the mesenteric lymph nodes of high dose animals. The incidence of neoplastic lesions in treated animals was comparable to controls. BARDAC 2280 was not carcinogenic in male or female rats.

	NOEL	LOEL
Males & Females	750 ppm	1500 ppm

The LOEL is based on increased incidence on nonneoplastic lesions in the mesenteric lymph nodes (sinusoidal blood, hemosiderosis and histiocytosis).

CORE CLASSIFICATION: Minimum

30

NEW ITEM

One-liner

CITATION

STUDY TYPE: Teratology - rats (83-3(a))

STUDY: Developmental toxicity valuation of didecyldimethyl-ammoniumchloride administered by gavage to CD (Sprague-Dawley) rats

LABORATORY: Bushy Run Research Center, Export PA

REPORT NO./DATE: 53-534/17 May 1991

EPA ACCESSION NO.: 418867-01

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 80.8% [ai]

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: In a developmental toxicity study, Sprague-Dawley rats were administered test compound daily by gavage at dose levels of 0, 1, 10 or 20 mg/kg/day on gestation days 6-15, inclusively. Maternal toxicity at 10 mg/kg/day included clinical signs (audible respiration). At 20 mg/kg/day, maternal animals exhibited clinical signs (audible respiration, gasping), decreased body weight gain and decreased food consumption during dosing period. No developmental toxicity was noted at the highest dose tested.

	NOEL	LOEL
Maternal	1 mg/kg/day	10 mg/kg/day
Developmental	20 mg/kg/day	not determined

CORE CLASSIFICATION: guideline

NEW ITEM

010689

One-liner

CITATION

STUDY: Developmental toxicity study of didecyldimethyl-ammoniumchloride administered by gavage to New Zealand white rabbits

LABORATORY: Bushy Run Research Center, Export PA

REPORT NO./DATE: 51-590/27 Jan 1989

EPA ACCESSION NO.: 410187-01

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 80% (ai)

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: In a developmental toxicity study, NZ white rabbits were administered test compound daily by gavage at dose levels of 0, 1, 3, or 10 mg/kg/day on gestation days 6-18, inclusively. At 3 and 10 mg/kg/day maternal animals exhibited clinical signs (hypoactivity, labored and/or audible respiration) and decreased body weight gain during dosing period. At 10 mg/kg/day there was increased incidence of mortality. Developmental toxicity included decreased fetal body weight and increased number of dead fetuses at 10 mg/kg/day.

	NOEL	LOEL
Maternal	1 mg/kg/day	10 mg/kg/day
Developmental	3 mg/kg/day	10 mg/kg/day

CORE CLASSIFICATION: Minimum

12

22

NEW ITEM

One-liner

CITATION

STUDY: Two-generation reproduction study in Sprague-Dawley (CD) rats with didecyldimethylammoniumchloride administered in diet

LABORATORY: Bushy Run Research Center, Export PA

REPORT NO./DATE: 52-648/1 Feb 1991

EPA ACCESSION NO.: 418045-01

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 80.8% (ai)

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: In a two-generation reproduction study, Sprague-Dawley (CD) rats were fed test compound at dietary levels of 0, 300, 750 or 1500 ppm (premating: 0, 20, 50, 100 mg/kg/day, males; 0, 24, 61, or 122 mg/kg/day, females). Parental toxicity at 1500 ppm consisted of decreased body weight/body weight gain. Reproductive toxicity at 1500 ppm consisted of decreased mean pup body weight/body weight gain during the postnatal period.

	NOEL	LOEL
Parental	750 ppm	1500 ppm
Reproductive	750 ppm	1500 ppm

CORE CLASSIFICATION: guideline

NEW ITEM

610689

One-liner

CITATION

STUDY TYPE: Mutagenicity: In vivo chromosomal aberration (84)

STUDY: Analysis of metaphase chromosomes obtained from bone marrow of rats treated with P0151

LABORATORY: Huntingdon Research Centre, Ltd, Huntingdon, UK

REPORT NO.(DATE): LZA 24/8761 (1 April 1987)

EPA ACCESSION NO.: 407058-02

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 50% (ai)

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: Negative for the induction of structural chromosomal aberrations in rat bone marrow.

CORE CLASSIFICATION: Unacceptable

24

UPDATE

One-liner

CITATION

STUDY TYPE: Mammalian cells (CHO) in culture cytogenicity assay (84-2)

STUDY: P0151: Chromosomal aberrations assay with chinese hamster ovary cells in vitro

LABORATORY: Inveresk Research International, Musselburgh, Scotland

REPORT NO./DATE: 737717/October 1986

EPA ACCESSION NO.: 412526-01

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 50% [ai]

P.C. CODE: 069149

CASWELL NO: 331A

RESULTS: Negative for the induction of chromosomal aberations in CHC cells.

CORE CLASSIFICATION: Acceptable

25

KEY ITEM

010684

One-liner

CITATION

STUDY TYPE: Forward gene mutation (CHO/HGPRT) (84-2)

STUDY: Mutagenicity test on didecyldimethylammoniumchloride (DDAC) in the CHO/HGPRT forward mutation assay

LABORATORY: Hazleton Laboratories, America, Kensington, MD

REPORT NO. (DATE): 10141-0-435 (9 Sept 1988)

EPA ACCESSION NO.: 408952-02

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 80% [ai]

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: Negative for the induction forward gene mutations in CHO cells at the HGPRT locus.

CORE CLASSIFICATION: Acceptable

UPDATE [redacted]

One-liner

CITATION

STUDY TYPE: Mutagenicity: UDS Assay in primary hepatocytes (84-4)

STUDY TITLE: Mutagenicity test on didecyldimethyl-ammoniumchloride in the rat primary hepatocyte assay

LABORATORY: Hazleton Laboratories America, Inc.

REPORT NO. (DATE): 10141-0-447 (12 Sept 1988)

EPA ACCESSION NO.: 408952-01

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 80% [ai]

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

UPDATE

016689

CITATION

STUDY TYPE: Metabolism - Rat (85-1)

STUDY TITLE: Main Study: Absorption, distribution, metabolism and excretion studies of didecyldimethylammoniumchloride (DDAC) in the rat.

Addendum: Absorption, distribution, metabolism and excretion studies of didecyldimethylammoniumchloride (BARDAC 22) in the rat.

LABORATORY: Bushy Run Research Center, Export PA

REPORT NO./DATE: P01421/1 Dec 1989 (Main study)
P01421/18 Dec 1989 (Addendum)

EPA ACCESSION NO.: 416171-01 (Main Study)
413851-01 (Addendum)

TEST MATERIAL: BARDAC 22

Didecyldimethylammoniumchloride

Purity: 99.4% (ai)

P.C. CODE: 069149

CASWELL NO.: 331A

RESULTS: The absorption, distribution, metabolism and excretion of ^{14}C-BARDAC was studied in male and female rats. For the single dose experiments, rats were orally gavaged with 10 mg/kg or 50 mg/kg; for the repeated low dose study animals were fed a diet containing unlabeled BARDAC 22 at 100 ppm in diet for 14 days, followed by single oral gavage dose of ^{14}C-BARDAC at 10 mg/kg of Day 15. Total recovery ranged from 90.8 to 100.9% of the administered dose. Fecal elimination predominated and accounted for 89.11 to 99.46% of the dose. Bioaccumulation of BARDAC 22 was low (< 1%). The proposed metabolic pathway for BARDAC 22 suggests that oxidization occurs at the two decyl side chains (probably at or near the terminal end) to form hydroxy and hydroxyketo derivatives. MS data indicated that 2-methyl substituents of BARDAC 22 remained unchanged. Less unchanged parent compound was present in the feces of females, suggesting a greater amount of metabolism. The extent to which biliary excretion and/or retention of parent compound in the gut contributed to the high fecal elimination could not be evaluated because an i.v. study was not performed.

CORE CLASSIFICATION: Supplementary (i.v. study needs to be done)

NEW ENTRY [redacted]

One-liner

CITATION

STUDY TYPE: 90-Day Feeding - Rats (§82-1)

TITLE: Ninety-day dietary toxicity study with alkyl dimethyl benzyl ammonium chloride (ADBAC)

LABORATORY: Bushy Run Research Center

REPORT NO./DATE: 51-503/20 June 1988

EPA ACCESSION NO.: 407466-01

TEST MATERIAL: ADBAC

Alkyl dimethyl benzyl ammonium chloride

Purity: 79.7% (ai)

P.C. CODE: 069105

CASWELL NO.: 016E

RESULTS: For 13 weeks, male and female rats were given diets containing 0, 100, 500, 1000, 4000, or 8000 ppm. The equivalent doses in mg/kg/day for the 100, 500, 1000, 4000 (estimated) and 8000 (estimated) ppm groups were 6.3, 31.2, 62.0, ≈ 248 and ≈ 496 for males and 0, 7.9, 38.3, 76.7, ≈ 308 and ≈ 616 for females. Treatment-related mortality was limited to the 4000 ppm (80% males and 73% females) and 8000 ppm (100% for males and females) groups. The 4000 ppm animals also showed decreased mean body weights, body weight gain and food consumption and increased incidence of gross and microscopic lesions.

	NOEL	LOEL
Males	500 ppm	1000 ppm
Females	1000 ppm	4000 ppm

The LOEL is based on decreased body weight and body weight gain in males and increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidence of gross and microscopic lesions in females.

CORE CLASSIFICATION: Guideline. This study satisfies guideline requirements (§82-1) for a 90-day feeding study in rats and is acceptable for regulatory purposes.

29

NEW ITEM

010689

One-liner

CITATION

STUDY TYPE: Developmental Toxicity - Rats (83-3)

STUDY TITLE: Teratologic evaluation of four quaternary compounds (Barquat MB-50; Barquat MX-50; Barquat 4250; Barquat 4250Z (an unpublished review of teratology studies submitted by Waverly Research Center, Food and Drug Research Laboratories, Inc)

LABORATORY: Waverly Research Center, Food and Drug Research Laboratories, Inc.

REPORT NO. (DATE): 5154 (11 Feb 1977)

EPA ACCESSION NO.: 228149

TEST MATERIAL	P.C. CODE	CASWELL NO.
Barquat MB-50	069105	016E
Barquat MX-50	069104	016C
BTC 741	069111	019
BTC 824	069154	019F

Barquat 4250 (25% Barquat MX-50 and 25% BTC 741)
Barquat 4250-Z (25% Barquat MX-50 and 25% BTC 824)

RESULTS: Four quaternary compounds were evaluated for potential of inducing developmental toxicity in Wistar albino rats following oral administration at 0, 10, 25, or 50 mg/kg/day during gestational days (GD) 6-15, inclusively. Based on the summary data, neither maternal nor developmental toxicity was observed in these studies. A lack of maternal toxicity at the highest dose level indicates an improper selection of that dose level. However, these conclusions cannot be verified since a lack of individual data and rationale for the selection of dose levels were not provided. In addition, compliance statements and analytical chemistry data were not submitted.

CORE CLASSIFICATION: Supplementary

3C

30

010689

NEW ENTRY

One-liner

CITATION

STUDY TYPE: Mutagenicity: Micronucleus assay (84-2)

TITLE: Series 84-2 assessment of the mutagenic activity of ityamine 3500 in the mouse nucleous test

LABORATORY: Scantox Laboratories, Ltd. Skensved, Denmark

REPORT NO.(DATE): 1075.3 (16 Dec 1985)

EPA ACCESSION NO.: 403111-01

TEST MATERIAL: ADBAC

Alkyl dimethyl benzyl ammonium chloride

Purity: 79.7% [ai]

P.C. CODE: 069105

CASWELL NO.: 016E

RESULTS: Negative

CORE CLASSIFICATION: Unacceptable

NEW ITEM

610689

One-liner

CITATION

STUDY TYPE: Mutagenicity: UDS Assay in primary hepatocytes (84-4)

STUDY TITLE: Genotoxicity test on alkyl dimethyl benzyl ammonium chloride (ADBAC) in the assay for unscheduled DNA synthesis in rat liver primary cell cultures

LABORATORY: Hazleton Washington, Inc.

REPORT NO. (DATE): 14778-0-447 (15 April 1992)

EPA ACCESSION NO.: 422908-01

TEST MATERIAL: ADBAC

Alkyl dimethyl benzyl ammonium chloride

Purity: 80% (ai)

P.C. CODE: 069105

CASWELL NO.: 016E

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

-2-

32

NEW ITEM

510639

One-liner

CITATION

STUDY TYPE: Mutagenicity: Gene mutation in CHO cells (84-2)

STUDY TITLE: Mutagenicity test on alkyl dimethyl benzyl ammonium chloride (ADBAC) in the CHO/HGPRT forward gene mutation assay

LABORATORY: Hazleton Laboratories America, Inc.

REPORT NO. (DATE): 10238-0-435 (23 Jan 1989)

EPA ACCESSION NO.: 410127-01

TEST MATERIAL: ADBAC

Alkyl dimethyl benzyl ammonium chloride

Purity: 80% [ai]

P.C. CODE: 069105

CASWELL NO.: 016E

RESULTS: Negative

CORE CLASSIFICATION: Acceptable

NEW ENTRY

010689

One-liner

CITATION

STUDY TYPE: Metabolism - Rat (§85-1)

TITLE: Absorption, distribution, metabolism and excretion of alkyl dimethyl benzyl ammonium chloride (ADBAC) in the rat

LABORATORY: Biological Test Center, Irvine, CA

REPORT NO./DATE: P01359/26 January 1989

EPA ACCESSION NO.: 409907-01

TEST MATERIAL: ADBAC

Alkyl dimethyl benzyl ammonium chloride

Purity: 79.7% [ai]

P.C. CODE: 069105

CASWELL NO.: 016E

RESULTS: The pharmacokinetic profile of ^{14}C-ADBAC was studied in male and female rats orally gavaged with low (10 mg/kg), high (50 mg/kg) or repeated low dose (100 ppm in diet for 14 days followed by single dose at 10 mg/kg) of test compound, additionally an i.v. low (10 mg/kg) dose study was also performed. Fecal elimination accounted for > 90%, while 5.8 to 7.8% appeared in the urine. For i.v. dosing 20 to 30% appeared in the urine with 44 to 55% in the feces. Tissue accumulation of orally-dosed animals negligible (< 1% of administered dose), while the carcasses and tissues of i.v. dosed animals retained 33.4% (males) and 35.8% (females) of the administered dose. Identification of metabolites were not performed in this study.

CORE CLASSIFICATION: Supplementary. This study does not satisfy guideline requirements (§85-1) for a metabolism study in rats and is not acceptable for regulatory purposes. The study may be upgraded to guideline if metabolic profile data are submitted and judged to be acceptable.

010689

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 18 Nov
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/18/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral - Rat (§81-1)

DP BARCODES: D188679, D189620 **SUBMISSION NO.:** S436173

P.C. CODE: 069207 **CASWELL NO.:** new chemical

MRID NO.: 424770-05

TEST MATERIAL: ISOBARDAC

SYNONYMS: BARDAC 2180
1-Decanaminium, N-isononyl-N,N-dimethyl-, chloride

STUDY NUMBER: 91-8357-21 (A)

SPONSOR: Lonza, Inc., Fair Lawn, NJ 07410

TESTING FACILITY: Hill Top Biolabs, Inc., Miamiville, OH 451[illegible]

TITLE OF REPORT: Acute Oral Toxicity in Rats - Median Lethal Dosage Determination with ISOBARDAC

AUTHOR: T.D. Morris

REPORT ISSUED: 5 March 1992

CONCLUSIONS: Animals were orally gavaged with test compound at doses of 50, 130, 200, 320 or 500 mg/kg body weight (doses not corrected for percent active ingredient).

Male and Female LD_{50}: 224 mg/kg (corrected for percent a.i.)
275 mg/kg (not corrected for a.i.)

Toxicity category II

CORE CLASSIFICATION - Guideline

This study satisfies guideline requirement (81-1) for an acute oral toxicity study in the rat and is acceptable for regulatory purposes.

3.

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(10689

A. MATERIALS

1. Test compound: ISOBARDAC Description: pale-yellow colored liquid Batch #: 91-0190P Purity: 81.5% [a.i.] Contaminants: not given

2. Test animals: Species: Rat Strain: Sprague-Dawley CD Age: not given Weight (g): 226 - 380 (males), 211 - 269 (females) Source: Harlan Sprague Dawley, Inc. Housing: Two animals in suspended cages Feed: Purina Laboratory Rodent Chow Water: Tap water, *ad libitum* Environment: Temperature, not given; Humidity, not given; Light cycle, 12 hr light/12 hr dark

B. METHODS

1. Study design: Animals (5/group/sex) were acclimated four days before initiation of the study. Following an overnight fast, animals were orally gavaged with test compound at doses of 50, 130, 200, 320 or 500 mg/kg body weight. Test compound was prepared as a 5% (w/v) concentration in distilled water. Doses were not corrected for percent active ingredient.

Animals were observed for signs of toxicity, moribundity and mortality several times during the day of dosing and twice daily, thereafter, for 14 days. Animals were weighed on days 0, 7, and 14 of the observation period. At the end of the observation period animals were necropsied for gross pathological examination.

2. Statistics: Means animal body weights and body weight gains were determined. The estimated LD_{50} was calculated using the method of Litchfield and Wilcoxon.

C. RESULTS AND DISCUSSION: Male and female rats were orally gavaged with test compound at doses of 50, 130, 200, 320 or 500 mg/kg body weight. Animals were observed twice daily for 14 days for signs of toxicity, mortality and moribundity; body weights were determined on study days 0, 7 and 14. A summary table for clinical observations was not provided with the study, however page 9 of the study states that "Clinical changes noted during the observation period included masticatory movements; slight emaciation; piloerection;wheezing, labored, and gasping breathing;abdominal region appeared bloated; hunched posture; slight to severe depression; unkempt fur; dirty hair coat; fecal, urine, and saliva stains; and reddish stains on muzzle, forepaws, nostrils, and around eyes." Body weight change was not affected by administration of test article. No remarkable changes related to the test material were observed in gross pathological examination.

Mortality data are summarized in Table 1, below. The LD_{50}, corrected for percent active ingredient, was determined to be 224 mg/kg body weight for both males and females.

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010689

Table 1: Cumulative Mortality Results (Data summarized from Table 1 of the study)

Dose[a] (mg/kg)	Study Day 0	1	2	3	4	7	14
Males							
500	0	5	--	--	--	--	--
320	0	2	2	3	3	3	3
200	0	0	1	1	1	1	1
130	0	0	0	0	0	0	0
50	0	0	1	1	1	1	1
Females							
500	0	5	--	--	--	--	--
320	0	4	4	4	4	4	4
200	0	0	1	1	1	1	1
130	0	1	1	1	1	1	1
50	0	1	1	1	1	1	1

[a] Not corrected for percent active ingredient

D. CONCLUSIONS: Animals were orally gavaged with test compound at doses of 50, 130, 200, 320 or 500 mg/kg body weight (doses not corrected for percent active ingredient).

Male and Female LD_{50}: 224 mg/kg (corrected for per)
275 mg/kg (not corrected fc a.i ,

Toxicity category II

CORE CLASSIFICATION - Guideline

This study satisfies guideline requirement (81-1) for an acute oral toxicity study in the rat and is acceptable for regulatory purposes.

010689

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 18 Nov 93.
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/18/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Primary Skin Irritation in Rabbits (§81-5)
DP BARCODES:	D188679, D189620 **SUBMISSION NO.:** S436173
P.C. CODE:	069207 **CASWELL NO.:** new chemical
MRID NO.:	424770-07
TEST MATERIAL:	ISOBARDAC
SYNONYMS:	BARDAC 2180 1-Decanaminium, N-isononyl-N,N-dimethyl-, chloride
STUDY NUMBER:	91-8357-21 (C)
SPONSOR:	Lonza, Inc., Fair Lawn, NJ 07410
TESTING FACILITY:	Hill Top Biolabs, Inc., Miamiville, OH 45147
TITLE OF REPORT:	Primary Skin Irritation Study in Rabbits with ISOBARDAC
AUTHOR:	T.D. Morris
REPORT ISSUED:	26 February 1992

CONCLUSIONS: Following a single application of test compound (0.5 ml) to the back of a single NZ white rabbit, erythema and edema developed early (30 min to 1 hr) and produced moderate to severe erythema (with necrosis) and severe edema after 24 hours.

TOXICITY CATEGORY I (Corrosive)

CORE CLASSIFICATION: Guideline

This study satisfies guideline requirement (§81-5) for a primary skin irritation study in rabbits and is acceptable for regulatory purposes.

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010689

A. MATERIALS

1. Test compound: ISOBARDAC Description: pale-yellow colored liquid Batch #: 91-0190P Purity: 81.5% [a.i.] Contaminants: not given

2. Test animals: Species: Rabbit Strain: NZ white Age: not given Weight (g): not given Source: LSR Ind. Housing: Singly in suspended cages Feed: Purina Laboratory Rabbit Chow Water: Tap water, *ad libitum* Environment: Temperature, not given; Humidity, not given; Light cycle, 12 hr light/12 hr dark

B. METHODS

1. Study design: The application sites of six male rabbit were prepared by clipping the fur from the scapular to lumbar region of the back. Approximately 0.5 ml of test material was applied to a 1-in² square gauze pad and placed on the exposed skin. The gauze was held in place with non-irritating tape and covered with an occlusive dressing. The test material remained in contact with the skin for 4 hours at which time the gauze pad was removed and the application site cleaned with water and wiped dry. The skin was examined at 30 min, 1 hr and daily, thereafter, for 3 days. Application sites were scored for edema and erythema using the Draize method.

2. Quality assurance: Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. RESULTS AND DISCUSSION: No deaths occurred during the study. Data are presented in Appendix 1. Well-defined erythema and severe edema developed between 30 min to 1 hr after removal of the test compound. Erythema was accompanied by discoloration and blanching extending beyond the application site. After 24 hours moderate to severe erythema with necrosis and severe edema were present. Because the test compound was corrosive, the experiment was limited to observation on one animal over a 24-hour time period.

D. CONCLUSIONS: Following a single application of test compound (0.5 ml) to the back of a single NZ white rabbit, erythema and edema developed early (30 min to 1 hr) and produced moderate to severe erythema (with necrosis) and severe edema after 24 hours.

TOXICITY CATEGORY I (Corrosive)

CORE CLASSIFICATION: Guideline

This study satisfies guideline requirement (§81-5) for a primary skin irritation study in rabbits and is acceptable for regulatory purposes.

Page 49 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 29Oct93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/13/93
Section IV, Tox. Branch II (H7509C)

5/0674

DATA EVALUATION REPORT

STUDY TYPE:	Photoallergy study - Guinea Pigs
DP BARCODES:	D188679 D189620
SUBMISSION NO.:	S436173
P.C. CODE:	069207
CASWELL NO.:	new chemical
MRID NO.:	424770-06
TEST MATERIAL:	ISOBARDAC
SYNONYMS:	BARDAC 2180 1-Decanaminium, N-isononyl-N,N-dimethyl-, chloride
STUDY NUMBER:	91-8357-21 (E)
SPONSOR:	Lonza, Inc., Fair Lawn, NJ 07410
TESTING FACILITY:	Hill Top Biolabs, Inc., Miamiville, OH 45147
TITLE OF REPORT:	Photoallergy Study in Guinea Pigs with ISOBARDAC
AUTHOR:	T.D. Morris
REPORT ISSUED:	26 February 1992

CONCLUSIONS: The study concluded that the test compound was not a photoallergen, however, the lack of a significant positive response indicates that the assay was not sensitive enough to detect weak photoallergens

CORE CLASSIFICATION: Unacceptable (Supplemental data, no guideline requirements)

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A. MATERIALS

1. Test compound: ISOBARDAC Description: pale-yellow colored liquid Batch #: 91-0190P Purity: ≥1.5% (a.i.) Contaminants: not given

2. Test animals: Species: Guinea Pig Strain: Hartley albino Age: not given Weight (g): 356 - 589 Source: Harlan Housing: Singly in suspended cages Feed: Purina Guinea Pig Chow Water: Tap water, *ad libitum* Environment: Temperature, not given; Humidity, not given; Light cycle, 12 hr light/12 hr dark

B. METHODS

1. Preparation of animals: One day before treatment, application sites were clipped free of hair and a cream depilatory applied. The skin was then thoroughly cleansed with water, dried and returned to their cages. Different application sites were used for the induction and challenge phases of the study.

2. Test material administration and irradiation: A 0.3 ml aliquot of test material or vehicle was applied to the pad of a 25 mm Hill Top Chamber®, which was occluded with rubber dental dam. Animals were restrained during the exposure period. Chambers were removed after approximately four hours. Animals in the non-irradiated test groups were returned to their cages, while those in the irradiated groups were exposed to fluorescent UVA and UVB light sources with radiation levels of 0.31 to 0.55 mw/cm^2 and 3.3 - 5.5 µw/cm^2, respectively. After a two-hour exposure, animals were returned to their cages.

2. Preliminary Dermal Irritation Study: A preliminary dermal irritation study was carried out to determine the concentration of test compound to be used in the induction and challenge phases of the study. Aqueous dilutions (w/v) of test compound were prepared at concentrations (v/v) of 0.05, 0.1, 0.25, or 0.5% for non-irradiated animals (2/sex), and 0.05, 0.1, or 0.25% for UV-irradiated animals (3/sex).

3. Main Study: Animals were randomly assigned to study groups as outlined in Table 1, below. During the induction phase of the study, animals were three times per week for three consecutive weeks. After a 10- to 14-day rest period, animals were challenged once with appropriate treatment. Skin sites were evaluated 24 and 48 hours after the challenge treatment and scored using the following scale: 0, no reaction; ±, slight, patchy erythema; 1, slight, but confluent or moderate, but patchy erythema; 2, moderate erythema; 3, severe erythema with or without edema.

4. Quality assurance: Quality assurance was documented by signed and dated GLP and quality assurance statements.

Table 1: Study Design (Taken from Table on page 38 of study)

Study Group	Number of Animals Male	Female	Induction	Challenge
1	5	5	0.1% TC[a] + UV	0.1% TC + UV
2	4	6	Untreated	0.1% TC + UV
3	5	5	1% TCSA[b] + UV	0.05% TCSA + UV
4	5	5	1% TCSA	0.05% TCSA
5	3	2	Untreated	0.05% TCSA + UV
6	2	3	Untreated	0.05% TCSA

[a] Test compound (TC) dissolved in distilled water to yield indicated percentages (w/v).
[b] Positive control material (3,3',4',5-tetrachlorosalicylanilide (TCSA) was dissolved in 95% ethanol for induction phase and acetone for challenge phase.

C. RESULTS AND DISCUSSION:

1. Preliminary study: The results of the preliminary studies are summarized in Appendix 1. The highest, non-irritating concentration was achieved at 0.1% for both irradiated and non-irradiated groups.

2. Main study: The results of the main study are presented in Appendix 2. In general, groups treated with test compound either had no response or exhibited slight, patchy erythema at the 24- and 48-hour observation times. A single animal in the irradiated naive control (Group 2) had a single incidence of a grade 1 response. The Group 3 (irradiated positive control) animals showed only 20% and 10% response with mean severity scores were 0.7 and 0.6 after 24 and 48 hours, respectively. These values were much lower than published values[1], where the incidence of positive responses was 80% with severity scores of 2.0 at 24 hours and 1.9 at 48 hours.

D. CONCLUSIONS: The study concluded that the test compound was not a photoallergen, however, the lack of a significant positive response indicates that the assay was not sensitive enough to detect weak photoallergens.

CORE CLASSIFICATION: Unacceptable (Supplemental data, no guideline requirements)

[1] Buehler, E.V., Newmann, E.A., and Parker, R.D., Use of the occlusive patch to evaluate the photosensitive properties of chemicals in guinea-pigs, *Fd. Chem. Toxicol.* 23: 689-694 (1985)

Page_____ is not included in this copy.

Pages 44 through 45 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

Reviewed by: Robert F. Fricke, Ph.D. 13 Oct 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. 11/19/93
Section IV, Tox. Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: 90-Day Feeding - Dogs (§82-1)

DP BARCODES: D188679, D189620 **SUBMISSION NO.:** S436173

P.C. CODE: 069149 **CASWELL NO.:** 331A

MRID NO.: 402629-01

TEST MATERIAL: BARDAC 22

SYNONYMS: Didecyldimethylammoniumchloride

STUDY NUMBER: 2224a

SPONSOR: Lonza, Inc., Fair Lawn, NJ

TESTING FACILITY: Food and Drug Research Laboratories, Inc.

TITLE OF REPORT: 90-Day feeding study in dogs with a quaternary ammonium sanitizer Bardac®-22

AUTHOR: D.E. Bailey

REPORT ISSUED: 7 April 1975

CONCLUSIONS: In a 90-day feeding study, male and female beagle dogs were given test compound at dosages of 0, 5, 15 or 50 mg/kg body weight/day. All animals survived to terminal sacrifice. Individual clinical signs were not recorded during the study. High-dose males and females experienced marked decrease in body weight gain, food consumption and food efficiency. Clinical chemistry, hematology, urinalysis, and pathology results did not reveal and treatment-related effects.

	NOEL	LOEL
Males and females	15 mg/kg/day	50 mg/kg/day

The LOEL is based on decreased body weight gain, food consumption and food efficiency.

CORE CLASSIFICATION: This study is classified as Supplementary since individual clinical signs and analytical data were not provided. This study is acceptable for regulatory purposes and satisfies the guideline requirement [§82-1(a)], since a 1-year oral study in this species (MRID No. 41970[illegible]-01) is available.

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A. MATERIALS

1. Test Compound: BARDAC-22 Description: clear yellow liquid Batch #: B-2754 Purity: 50% [ai] Contaminants: not given

2. Test animals: Species: Dog Strain: Beagle Age: 36 - 65 weeks Weight (kg): 8.4 - 13.0 (males), 6.5 - 10.9 (females) Source: Closed breeding colony of Waverly Division, Food and Drug Research Laboratory, Inc. Housing: Individually in mesh-bottom cages Feed: Purina Dog Chow Water: Tap water, *ad libitum* Environment: Air conditioned (temperature, humidity and light cycle not given)

B. METHODS

1. Animal Assignment: Animals were assigned randomly to main study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group		Dosage (mg/kg/day)	Animals/Group	
			Male	Female
Control	(CON)	0	4	4
Low	(LDT)	5.0	4	4
Mid	(MDT)	15.0	4	4
High	(HDT)	50.0	4	4

2. Diet Preparation: An appropriate amount of test compound for each dosage level was dissolved in acetone and mixed with basal diet. The test diet was thoroughly mixed and air dried. Control diet was prepared in a similar manner using acetone and basal diet.

3. Statistical Evaluations: Sample means were calculated; no other statistical procedures were indicated.

C. REGULATORY COMPLIANCE

1. This study was performed in 1974/1975. This was prior to Good Laboratory Practices standards. The quality and quantity of the data reported are considered by this reviewer to be sufficient to evaluate the study.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS

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1. Analysis of Test Diet: Not performed

2. Observations: Frequency of observations not stated.

   a. Clinical observations: Individual clinical signs were not recorded.

   b. Mortality (survival): All animals survived until the scheduled sacrifice.

3. Body Weight Gain: Animals body weights were measured at the start of the study, at weekly intervals during the study and at terminal sacrifice. The mean body weight gains over the entire study (Weeks 1 to 13) are summarized in Table 2, below. Treatment-related effects were noted in both the high-dose males and females.

NOTE: The value for body weight gain for control males (Study Table 2, page 11) is incorrectly stated as 3.0 kg, the correct value is 1.73 kg).

4. Food Consumption and Food Efficiency: A weighed amount (700 g) of dried test diet was presented to the animals for one hour per day for six days. Any food not consumed within the one hour period was removed from the cage and weighed.

   a. Food consumption results: The study tabulated the weekly food consumption over the 13 week study. The average weekly food consumption data (calculated by the reviewer) are summarized in Table 2, below. For the high-dose animals, food consumption was markedly less than controls.

   b. Food efficiency results: Food efficiency was calculated based on the mean body weight gain and the average weekly food consumption (Table 2). Food efficiency for the high-dose males was more than an order of magnitude less than controls; females showed a negative food efficiency value.

5. Ophthalmological examinations: Examinations were performed during the prestudy acclimation period and again before terminal sacrifice. No treatment-related eye lesions were observed.

6. Clinical Pathology: Clinical chemistry and hematology was performed before the start of the study to establish baseline values and again after 30 and 90 days of treatment.

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Table 2: Body Weight Gain, Food Consumption and Food Efficiency

Observation	Sex	COM	LDT	MDT	HDT
Body Weight Gain[a] (Weeks 1 - 13)	Males	1.73	1.35	1.70	0.18
	Females	1.40	1.53	1.35	0.07
Food Consumption[a] (kg/week)	Males	4.184	4.167	4.182	3.640
	Females	3.722	4.067	3.845	3.096
Food Efficiency[b]	Males	0.412	0.324	0.406	0.032
	Females	0.377	0.373	0.349	-0.011

a Calculated by reviewer from data presented in Appendix III of study

b Calculated by reviewer from body weight gain and food consumption data. Units = kg body weight gain/kg food consumed

a. Hematology: The following checked (X) hematology parameters were examined:

X Hematocrit (HCT)
X Hemoglobin (HGB)
X Leukocyte count (WBC)
X Erythrocyte count (RBC)
Prothrombin time
X Leukocyte differential count
Mean corpuscular HGB (MCH)
Mean corpuscular HGB conc. (MCHC)

Results: Hematological evaluation of control and treated dogs did not reveal any treatment-related effects.

b. Clinical Chemistry: The following checked (X) clinical chemistry parameters were examined:

Electrolytes
Calcium (Ca)
X Chloride (Cl)
X Sodium (Na)
Phosphorous (P_i)
X Potassium (K)

Enzymes
γ-Glutamyl transpeptidase (GGT)
X Alkaline phosphatase (ALK)
X Aspartate aminotransferase (SGOT/AST)
X Alanine aminotransferase (SGPT/ALT)

Other
X Glucose (GLU)
Blood creatinine (CREAT)
X Blood urea nitrogen (BUN)
Phospholipid (PL)
X Total bilirubin (TBIL)
Direct bilirubin (DBIL)
Indirect bilirubin (IBIL)
X Protein, total (PROT)
Triglycerides (TG)
Albumin (ALB)

Results: No significant treatment-related changes were noted in any of the clinical chemistry parameters measured.

7. Urinalysis: Urinalysis was performed before the start of study and again after 30 and 90 days of treatment. The following checked (X) parameters were examined:

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X	Color	X	Glucose
X	Specific gravity	X	Ketone Bodies
X	Protein		Bi'e Pigments
X	Appearance		Urobilirubin
X	Sediment	X	Total Bilirubin
X	pH	X	Occult Blood

Results: No treatment-related effects were noted in any of the animals.

8. Sacrifice and Pathology: Detailed pathological examination was performed on male and female animals in the control and treatment groups. The checked (X) tissues were fixed in 10% neutral buffered formalin; the checked (XX) organs of control and high-dose animals were examined histologically. Selected organs (CAPITAL LETTERS) were also weighed.

Digestive system		Cardiovas./Hematol		Neurologic	
	Tongue		Aorta	X	BRAIN
	Salivary glands	XX	HEART	X	Periph. nerve
	Esophagus	X	Bone marrow	X	Spinal cord
XX	Stomach	XX	Lymph nodes	X	PITUITARY
XX	Duodenum	XX	SPLEEN	XX	Eyes
XX	Jejunum		Thymus	Glandular	
XX	Ileum	Urogenital		XX	ADRENALS
XX	Cecum	XX	KIDNEYS		Lacrimal gland
XX	Colon	XX	Urinary bladder		Mammary gland
	Rectum	XX	TESTES		Parathyroids
XX	LIVER		Epididymides	XX	THYROIDS
X	Gallbladder	X	PROSTATE	Other	
XX	Pancreas		Seminal vesicle		Bone
Respiratory		XX	OVARIES		Skeletal muscle
	Trachea	X	Uterus	X	Skin
X	Lungs		Vagina	XX	Gross lesions
	Nasal Passages		Cervix		
	Larynx				

a. Organ Weights: No treatment-related effects were noted in either the absolute or relative organ weights. All values were comparable to controls.

b. Gross Pathology: Gross examination of tissues taken at the terminal sacrifice did not reveal any treatment-related abnormalities.

c. Histopathology: Histopathological examination of animals in the control and high-dose group did not reveal any changes which could be attributable to treatment.

E. DISCUSSION: In a 90-day feeding study, male and female beagle dogs were given test compound at dosages of 0, 5, 15 or 50 mg/kg body weight/day. All animals survived to terminal sacrifice. Individual clinical signs were not recorded during

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the study. High-dose males and females experienced marked decrease in body weight gain, food consumption and food efficiency. Clinical chemistry, hematology, urinalysis, and gross and histopathology results did not reveal and treatment-related effects.

	NOEL	LOEL
Males and females	15 mg/kg/day	50 mg/kg/day (HTD)

The LOEL is based on decreased body weight n, food consumption and food efficiency.

CORE CLASSIFICATION: This study is classified as Supplementary since individual clinical signs and analytical data were not provided. This study is acceptable for regulatory purposes and satisfies the guideline requirement [§82-1(a)], since a 1-year oral study in this species (MRID No. 419704-01) is available.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 13 Oct 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. 11/8/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	90-Day Feeding - Rats (82-1)
DP BARCODES:	D188679, D189620 **SUBMISSION NO.:** S436173
P.C. CODE:	069149 **CASWELL NO.:** 331A
MRID NO.:	409663-02
TEST MATERIAL:	BARDAC 2280
SYNONYMS:	Didecyldimethylammoniumchloride
STUDY NUMBER:	51-506
SPONSOR:	Lonza, Inc., 22-10 Route 208, Fair Lawn, NJ
TESTING FACILITY:	Bushy Run Research Center, R.D. #4, Mellon Road, Export, PA
TITLE OF REPORT:	Ninety-day subchronic oral toxicity study with didecyldimethylammoniumchloride
AUTHOR:	J.P. Van Miller
REPORT ISSUED:	6 September 1988

CONCLUSIONS: For 13 weeks, male and female rats were given diets containing 0, 100, 300, 600, 1000, or 3000 ppm (respective mg/kg/day equivalents: 0, 6.2, 18.5, 36.8, 60.7 and 175.4 for males; 0, 7.5, 22.3, 44.4, 74.3 and 225.5 for females). High-dose animals showed increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidence of gross pathological observations and non-neoplastic lesions. From the results of this study, NOEL and LOEL are:

	NOEL	LOEL
Males	1000 ppm (60.7 mg/kg/day)	3000 ppm (175.4 mg/kg/day)
Females	1000 ppm (74.3 mg/kg/day)	3000 ppm (225.5 mg/kg/day)

CORE CLASSIFICATION: Guideline. This study satisfies guideline requirements (§82-1) for a 90-day feeding study in rats and is acceptable for regulatory purposes.

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A. MATERIALS:

1. Test compound: BARDAC 2280 Description: viscous, honey-colored liquid Batch #: B-1889 Purity: 60.8% [a.i.]
Contaminants: not given

2. Test animals: Species: Rat Strain: Sprague-Dawley CD Age: 8 weeks Weight (g): 241.2 - 308.7 (males), 166.6 - 211.1 (females) Source: Charles River Breeding Laboratories, Inc., Portage, MI. Housing: Individually in suspended cages Feed: Purina Certified Rodent Chow #5002 Water: Tap water, *ad libitum* Environment: Temperature, 66 - 75 °F; Humidity, 20 - 40%; Light cycle, 12 hr light/12 hr dark

B. METHODS:

1. Study Design: For 90 days, male and female rats were exposed to test compound at dietary concentrations of 0, 100, 300, 600, 1000, or 3000 ppm. Animals were randomly assigned to study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Study Group		Dose in Diet* (ppm)	Animals/Group Male	Female
Control	(CON)	0	15	15
Low	(LDT)	100	15	15
Mid1	(MDT1)	300	15	15
Mid2	(MDT2)	600	15	15
Mid3	(MDT3)	1000	15	15
High	(HDT)	3000	15	15

* Adjusted for percent purity of active ingredient

2. Diet preparation: A concentrated premix was prepared by thoroughly mixing test compound with basal diet. The amount of test compound added was adjusted for percent purity of active ingredient. The 3000 ppm diet was prepared by dilution of the premix with basal diet. The remaining diets were prepared by serial dilution of the next higher concentration diet with basal diet.

3. Statistical Evaluations: Parametric data were initially analyzed for homogeneity of variances using Levene's test. Homogeneous data were further analyzed using analysis of variance (ANOVA). Data sets yielding a significant ANOVA result were further analyzed using pooled variance t-tests. Heterogeneous data sets were analyzed using ANOVA for unequal variances followed by separate variance t-tests. Nonparametric data were analyzed using Kruskal-Wallis' test or a modified (Mann-Whitney) Wilcoxon rank sum test. The Fisher's exact test was used to analyze frequency data.

C. REGULATORY COMPLIANCE

1. Quality assurance was documented by signed and dated GLP and quality assurance statements.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS:

1. Analysis of Diet: The prepared diets were analyzed for stability, homogeneity and concentration. Test diets were stable for at least 20 days at room temperature. Analysis of test diet samples, taken from the top, middle and bottom, indicated that the test compound was homogeneously distributed (coefficient of variation 1.4 to 4.4%) and within 87.8 and 109% of nominal concentrations. The concentration of test compound in the diet were also confirmed periodically throughout the study; concentrations were all within 93 to 106.6% of the nominal concentration.

2. Clinical Observations and Mortality: Animals were inspected twice daily for signs of toxicity, moribundity and mortality. A detailed clinical exams were performed once each week. Treatment-related deaths and moribund sacrifices were limited to the high-dose animals 12 males and 12 females were either found dead or were sacrificed *in extremis* (Table 2). Emaciation, perineal staining, pallor and loose feces were observed in the high-dose animals.

3. Body Weight and Body Weight Gains: Animals were weighed at the start of the study, weekly, thereafter, and at terminal sacrifice.

a. Body weight (Appendix 1): Significant, treatment-related decreases in mean body weights were noted only in the high-dose animals. The mean body weights of males were significantly decreased throughout the entire study. Female body weights were also decreased throughout the study, with statistical significance occurring only during Weeks 1, 2, and 10. At terminal sacrifice, high-dose males and females had body weights of 324 and 220 g, respectively, which were significantly ($p \leq 0.01$) lower than the control values of 515 and 285 g, respectively.

b. Body weight gains: Body weight gain data are presented in Appendix 2. Again, significant, treatment-related decreases in body weight gain were noted only in the high-dose animals. The weight gain

Table 2: Incidence of Clinical Observations (Data summarized from Table 1 of the study)

Observation	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
Number of animals	♂/♀	15	15	15	15	15	15
Found dead	♂	0	0	0	0	0	8
	♀	0	0	1	0	0	9
Moribund sacrifice	♂	0	0	0	0	0	4
	♀	0	0	0	0	0	3
Emaciation	♂	2	0	0	1	0	15
	♀	0	0	1	0	0	15
Perineal staining	♂	0	0	0	0	0	7
	♀	0	0	1	0	0	7
Pallor	♂	0	0	0	0	0	8
	♀	0	0	0	0	0	7
Loose Feces	♂	0	0	0	0	0	15
	♀	0	0	1	0	0	15

by males and females was depressed throughout the entire study. Males showed significant decreases during the entire study and females, only for the intervals from Week 0 through Weeks 2, 5, 9, 10, 11, 12, and 13.

4. Food Consumption and Achieved Compound Intake: Food consumption was measured at weekly intervals.

a. Food consumption: Food consumption was monitored at weekly intervals throughout the study (Appendix 3). Treatment-related effects were confined to the high-dose males and females. Through Week 12 of the study, high-dose males showed significantly lower food consumption than controls. High-dose females, on the other hand, showed an initial decrease in food consumption through Week 3, followed a consumption rate comparable to that of controls.

b. Achieved compound intake: The mean compound intake is summarized in Table 3, below.

5. Ophthalmological Examinations: Examinations were performed on all animals before the start of the study and at terminal sacrifice. During the prestudy examination, approximately 90% of the animals (equally distributed between all study groups) showed corneal crystals. At terminal sacrifice 85% were still affected. Since this finding was present before the study was started, it is clearly not treatment-related. Other sporadic ocular

findings were noted, but were not considered to be treatment-related.

Table 3: Compound Intake (Data summarized from Tables 5 and 6 of study)

Dose in Diet (ppm)	Compound Intake (mg/kg/day) Male	Female
100	6.2	7.5
300	18.5	22.3
600	36.8	44.4
1000	60.7	74.3
3000	175.4	225.5

6. Clinical Pathology: At terminal sacrifice, clinical chemistry and hematological analyses were performed on fasted animals. Analyses were performed on all high-dose animals and ten animals per sex, randomly selected from each of the treatment and control groups.

a. Serum chemistry results: The following parameters were evaluated:

Electrolytes
- Calcium (Ca)
- Chloride (Cl)
- Sodium (Na)
- Phosphorous (P_i)
- Potassium (K)

Enzymes
- γ-Glutamyl transpeptidase (GGT)
- Alkaline phosphatase (ALK)
- Aspartate aminotransferase (SGOT/AST)
- Alanine aminotransferase (SGPT/ALT)

Other
- Glucose (GLU)
- Blood creatinine (CREAT)
- Blood urea nitrogen (BUN)
- Phospholipid (PL)
- Total bilirubin (TBIL)
- Direct bilirubin (DBIL)
- Indirect bilirubin (IBIL)
- Protein, total (PROT)
- Triglycerides (TG)
- Albumin (ALB)
- Globulins (GLOB)
- A/G ratio (A/G)

Results: Significant, treatment-related changes in clinical chemistry parameters were limited to high-dose animals and consisted of decreased glucose, protein, albumin and globulins and increased phosphorous (Table 4). Other significant findings were observed, but were sporadic in nature and did not appear to be treatment-related.

b. Hematology results: The following parameters were evaluated:

- Hematocrit (HCT)
- Hemoglobin (HGB)
- Leukocyte count (WBC)
- Erythrocyte count (RBC)
- Platelet count (PLAT)
- Reticulocyte count (RC)
- Leukocyte differential count (DIF)
- Mean corpuscular HGB (MCH)
- Mean corpuscular HGB conc. (MCHC)
- Mean corpuscular volume (MCV)

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Results: Significant hematological effects, observed only in high-dose males, consisted of slight elevations in erythrocyte count, hemoglobin concentration and hematocrit (Table 5). Hematological results of females were not significantly different from control values.

Table 4: Clinical Chemistry Results (Data summarized from Appendix 3, Tables 1 and 2 of study)

Parameter	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
GLU (g/l)	♂	1.19	1.30	1.19	1.17	1.22	0.93**
	♀	1.25	1.24	1.14	1.21	1.22	0.94**
PROT (g/l)	♂	65	67	67	66	67	58*
	♀	70	68	65**	68	66*	56***
P_i (mg/l)	♂	60	56	58	57	58	86***
	♀	52	54	50	52	56	76**
ALB (g/l)	♀	37	36	35	37	36	30***
GLOB (g/l)	♀	33	32	30**	31	30*	26***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 5: Hematology Results (Data summarized from Appendix 3, Table 5 of study)

Parameter	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
RBC ($10^6/\mu l$)	♂	8.4	8.7	8.5	8.5	8.5	9.3**
HGB (g/dl)	♂	15.5	15.7	15.4	16.0	15.8	17.2***
HCT (%)	♂	43.8	44.7	44.0	45.2	45.0	49.6***

** $p \leq 0.01$, *** $p \leq 0.001$

7. Sacrifice and Pathology: Detailed pathological examination was performed on animals in the control and treatment groups. The tissues listed below were collected at necropsy and saved in 10% neutral buffered formalin. Histological examinations were performed on tissues (marked X and XX) from ten randomly selected animals of the control and 1000 ppm groups; for the remaining intermediate dose groups, the organs marked XX were examined from ten randomly selected animals in each dose group. Selected tissues (capital letters) were also weighed.

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Digestive system	Cardiovas./Hematol	Neurologic
X Pancreas	X Aorta	X BRAIN
X Salivary glands	X Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
XX Stomach	X Lymph nodes	X Pituitary
XX Duodenum	X Spleen	Eyes
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	X ADRENALS
X Cecum	XX KIDNEYS	Lacrimal gland
X Colon	X Urinary bladder	Mammary gland
X Rectum	X TESTES	X Parathyroids
XX LIVER	X Epididymides	X Thyroids
Respiratory	X Prostate	Other
X Trachea	Cervix	XX Gross lesions
X Lungs	Vagina	Skin
	X OVARIES	X Skeletal muscle
	X Uterus	Bone

a. Organ weights: Absolute and relative organ weights were measured at terminal sacrifice. Significant findings were again limited to animals in the high-dose group (Table 6). The absolute liver and kidney weights were significantly lower than controls. Although significant increases were noted in the relative brain, adrenal and testes weights of the high-dose animals, the differences appeared to be a reflection of the decreased terminal body weights rather than a treatment-related effect.

Table 6: Absolute and Relative Organ Weights (Data summarized from Tables 11, 12, 13, 14 and 15 of study)

Observation	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
Absolute Organ Weights (g)							
Liver	♂	14.9	14.4	15.3	14.7	15.5	9.38-
	♀	8.67	8.29	8.02-	8.36	8.70	7.21-
Kidney	♂	4.01	3.94	4.04	4.06	4.08	2.76-
	♀	2.36	2.31	2.34	2.43	2.40	1.97-
Relative Organ Weight (% of Terminal Body Weight)							
Brain	♂	0.409	0.412	0.421	0.416	0.403	0.595-
	♀	0.687	0.671	0.683	0.663	0.670	0.829-
Adrenals	♂	0.012	0.011	0.012	0.012	0.012	0.023-
Testes	♂	0.677	0.687	0.707	0.717	0.681	1.086-
Relative Organ Weight (% of Brain Weight)							
Liver	♂	714	683	723	703	734	487-
Kidneys	♂	192	187	190	195	194	144-
Adrenals	♂	2.91	2.73	2.80	2.89	2.99	3.81-

** $p < 0.01$

b. Gross pathology: Gross pathological examinations were performed on animals found dead or sacrificed in moribund condition during the study and surviving animals at terminal sacrifice. Treatment-related gross observations are summarized in Table 7, below. Findings were limited to high-dose groups where emaciation, hemorrhagic stomachs, decreased spleen size and dilated and distended cecum with abnormal contents were noted in both males and females.

Table 7: Incidence of Gross Pathological Observations (Data summarized from Appendix 2, Tables 1 to 4 of the study)

Observation	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
Moribund Sacrifices and Deaths							
Emaciation	♂	0/0	0/0	0/0	0/0	0/0	11/12
	♀	0/0	0/0	1/1	0/0	0/0	8/12
Stomach, Hemorrhage	♂	0/0	0/0	0/0	0/0	0/0	5/12
Spleen, Decreased size	♂	0/0	0/0	0/0	0/0	0/0	9/12
	♀	0/0	0/0	0/1	0/0	0/0	6/12
Cecum, Dilated/distended	♂	0/0	0/0	0/0	0/0	0/0	12/12
	♀	0/0	0/0	0/1	0/0	0/0	9/12
Cecum, Abnormal contents	♂	0/0	0/0	0/0	0/0	0/0	11/12
	♀	0/0	0/0	0/1	0/0	0/0	7/12
Terminal Sacrifice							
Emaciation	♂	0/15	0/15	0/15	1/14	0/15	2/3
	♀	0/15	0/15	0/14	1/14	0/15	1/3
Cecum, Dilated/distended	♂	0/15	0/15	0/15	0/15	0/15	2/3
	♀	0/15	0/15	0/14	0/14	0/15	3/3
Cecum, Abnormal contents	♂	0/15	0/15	0/15	0/15	0/15	2/3
Mesenteric Lymph Nodes, Color change							
Diffuse	♂	0/15	0/15	0/15	0/15	0/15	1/3
Focal/multifocal	♀	0/15	0/15	0/14	0/15	0/15	2/3

c. Microscopic pathology: Significant, treatment-related non-neoplastic lesions were observed only in the high-dose groups. High-dose males and females showed a higher incidence of glycogen depletion in the liver and contracted spleens. Additionally, high-dose females showed sinus erythrocytosis and lymphoid hyperplasia of the mesenteric lymph nodes. Other, lesions were observed, however, their occurrences were not dose-related and appeared to be sporadic in nature.

D. DISCUSSION: For 13 weeks, male and female rats were given diets containing 0, 100, 300, 600, 1000, or 3000 ppm (respective mg/kg/day equivalents: 0, 6.2, 18.5, 36.8, 60.7 and 175.4 for males; 0, 7.5, 22.3, 44.4, 74.3 and 225.5 for females).

Table 8: Incidence of Microscopic Observations (Data summarized from Appendix 2, Tables 5 and 6 of the study)

Observation	Sex	CON	LOW	MID1	MID2	MID3	HIGH
Moribund Sacrifices and Deaths							
Liver, Glycogen depletion	♂	0/0	0/0	0/0	0/0	0/0	7/7
	♀	0/0	0/0	0/0	0/0	0/0	8/10
Spleen, Contracted	♂	0/0	0/0	0/0	0/0	0/0	6/6
	♀	0/0	0/0	0/0	0/0	0/0	2/2
Terminal Sacrifice							
Cecum, Typhlitis	♂	0/10	0/0	0/0	0/0	0/10	3/3
	♀	0/10	0/0	0/0	0/0	0/10	3/3
Lymph nodes, mesenteric							
Sinus erythrocytosis	♀	0/10	0/0	0/0	0/0	2/10	2/2*
Lymphoid hyperplasia	♀	0/10	0/0	0/0	0/0	0/10	2/2*
All Deaths Combined							
Liver, Glycogen depletion	♂	0/10	0/10	0/10	0/10	0/10	7/10**
	♀	0/10	0/10	0/10	0/10	0/10	5/10*
Spleen, Contracted	♂	0/10	0/0	0/1	0/0	0/10	6/6**
	♀	0/10	0/0	0/1	0/0	0/10	2/2*
Lymph nodes, mesenteric							
Sinus erythrocytosis	♀	0/10	0/0	0/0	0/0	2/10	2/2*
Lymphoid hyperplasia	♀	0/10	0/0	0/0	0/0	0/10	2/2*

* $p \leq 0.05$, ** $p \leq 0.01$

Treatment-related lethality was limited to high-dose animals, where 80% mortality occurred within the first three weeks of the study. Adverse clinical signs included a high incidence of emaciation, perineal staining, pallor and loose feces in the high-dose animals. High-dose animals also had significantly lower mean body weights and decreased food consumption.

At terminal sacrifice, abnormal hematology and clinical chemistry results were noted in high-dose animals. Dose-related effects included increased erythrocyte counts, hemoglobin concentration and hematocrits in males, decreased serum glucose and protein and increased phosphorous concentrations in males and females, and decreased serum albumin and globulin in females.

Treatment-related gross and histopathological lesions were limited to high-dose animals only. Gross observations included emaciation, hemorrhagic stomachs, decreased spleen size and dilated and distended cecum with abnormal contents. Significant, non-neoplastic lesions included a higher incidence of glycogen depletion in the liver and splenic contraction in males and females and sinus erythrocytosis and lymphoid hyperplasia of the mesenteric lymph nodes in females. No neoplastic lesions were noted.

The LOEL is based on increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidences of gross pathological observations and non-neoplastic

lesions. From the results of this study, NOEL and LOEL are as follows:

	NOEL	LOEL
Males	1000 ppm (60.7 mg/kg/day)	3000 ppm (175.4 mg/kg/day)
Females	1000 ppm (74.3 mg/kg/day)	3000 ppm (225.5 mg/kg/day)

Core Classification: Guideline

This study satisfies guideline requirements (§82-1) for a 90-day feeding study in rats and is acceptable for regulatory purposes.

Page _____ is not included in this copy.

Pages 62 through 67 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

Reviewed by: Robert F. Fricke, Ph.D. Robert F Fricke 13 Dec 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 12/14/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Dermal - Rats (82-3)

DP BARCODES: D188679, D189620 SUBMISSION NO.: S436173

P.C. CODE: 069149 CASWELL NO.: 331A

MRID NO.: 413059-01

TEST MATERIAL: BARDAC 2280

SYNONYMS: Didecyldimethylammoniumchloride

STUDY NUMBER: 51-554

SPONSOR: Lonza Inc., 22-10 Route 208, Fair Lawn, NJ

TESTING FACILITY: Bushy Run Research Center, R.D. #4, Mellon Road, Export, PA

TITLE OF REPORT: Ninety-day subchronic dermal toxicity study with didecyldimethylammoniumchloride in rats

AUTHOR: M.W. Gill and J.P. Van Miller

REPORT ISSUED: 7 October 1988

CONCLUSIONS: Male and female Sprague-Dawley rats received repeated dermal dosing of test compound at 0, 2, 6, or 12 mg/kg/day for 6 hours/day, 5 days/week for 13 weeks. The dermal toxicity of test compound was evaluated in male and female Sprague-Dawley rats exposed at 0, 2, 6, or 12 mg/kg/day for 6 hours/day, 5 days/week for 13 weeks. No treatment-related effects were noted in mortality, weight gain, food consumption, or systemic toxicity. Toxicity was limited to treated skin of mid-dose females and high-dose males and females. The clinical and gross findings (erythema, edema, exfoliation, excoriation and ulceration) were confirmed by histopathological examination, where increased incidence of hyperkeratosis, acanthosis, epidermitis, dermatitis and ulceration were noted.

From the results of this study, NOELs and LOELs for systemic and dermal toxicity are as follows:

Toxicity		NOEL	LOEL
Systemic		> 12 mg/kg/day (HDT)	
Dermal	Males	6 mg/kg/day	12 mg/kg/day
	Females	2 mg/kg/day	6 mg/kg/day

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The dermal LOEL is based is based increased incidence of histopathological lesions (hyperkeratosis, males and females and acanthosis, epidermitis, dermatitis and ulceration in females).

CORE CLASSIFICATION: Minimum. This study satisfies guideline requirements (§82-3) for a 90-day dermal toxicity study in rats and is acceptable for regulatory purposes. Although systemic toxicity was not noted in this study, a systemic LOEL was established in a 90-day oral study in rats (MRID No.: 409663-02).

A. MATERIALS

1. Test compound: BARDAC 2250 Description: viscous, honey-colored liquid Batch #: B-1889 Purity: 80.8% [a.i.] Contaminants: not given

2. Test animals: Species: Rat Strain: Sprague-Dawley CD Age: 8 weeks Weight (g): 221.3 - 264.5 (males), 166.8 - 219.1 (females) Source: Charles River Breeding Laboratories, Inc., Portage, MI. Housing: Individually in suspended cages Feed: Purina Certified Rodent Chow #5002 Water: Tap water, *ad libitum* Environment: Temperature, 66 - 75 °F; Humidity, 20 - 40%; Light cycle, 12 hr light/12 hr dark

B. METHODS

1. Prestudy Acclimation: Healthy animals were acclimated to the cages for two weeks prior to the start of the study. Eight days before the start date, the fur on the backs of the animals was clipped. One day after clipping, all animals were wrapped in an occlusive dressing, as discussed below, for six hours a day for four days. Only animals which adapted to the dressing and had intact and normal skin on gross examination were placed on the study.

2. Preparation of Skin and Dosing: Animals were reclipped before the initial dosing, as needed during the study, and each Friday. Test compound was applied directly to the back and covered with sterile gauze, which was held in place with an occlusive dressing. After a six hour exposure period, the dressing was removed and the application site cleaned with water and dried. Animals were exposed five days per week for 13 weeks.

3. Animal assignments: Animals were randomly assigned to study test groups as shown in Table 1. Based on the application volume of 2 ml/kg, the equivalent dosage in mg/kg/day was determined.

Table 1: Animal Assignment to Study Groups

Study Group	Concentration* % (w/w)	Dosage (mg/kg/day)	Animals/Group Male	Female
Control (CON)	0	0	15	15
Low (LDT)	0.1	2	15	15
Mid (MDT)	0.3	6	15	15
High (HDT)	0.6	12	15	15

* Adjusted for percent purity of active ingredient

4. Dose preparation: Aqueous solutions of test compound were prepared to yield final concentrations of 0, 0.1%, 0.3%, or 0.6% (w/w). An appropriate amount of test compound

(adjusted for percent of active ingredient) was dissolved in water to yield the 0.6% solution. The mid and low dose solutions were prepared by serial dilution of the 0.6% solution. The prepared solutions were analyzed for stability, homogeneity and concentration. The test solutions were stable for at least 14 days at room temperature.

5. Statistical Evaluations: Parametric data were initially analyzed for homogeneity of variances using Levene's test. Homogeneous data were further analyzed using analysis of variance (ANOVA). Data sets yielding a significant ANOVA result were further analyzed using pooled variance t-tests. Heterogeneous data sets were analyzed using ANOVA for unequal variances followed by separate variance t-tests. Nonparametric data were analyzed using Kruskal-Wallis' test or the Wilcoxon rank sum test as modified by Mann-Whitney. The Fisher's exact test was used to analyze frequency data.

C. REGULATORY COMPLIANCE

1. Quality assurance was documented by signed and dated GLP and quality assurance statements.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS

1. Analysis of Test Diet: Analysis of test solution samples, taken from the top, middle and bottom of the mixing vessel, indicated that the test compound was homogeneously distributed (mean coefficient of variation 0.7 to 1.8%); the mean nominal concentrations ranged from 94.8 to 96.1%. The concentration of test compound was verified on a weekly basis for the first four weeks of the study and during weeks 8 and 13. Solutions were all within 94.2 and 108% of the nominal concentration.

2. Clinical Observations and Mortality: Animals were inspected once daily for signs of toxicity and twice daily for moribundity and mortality. A detailed clinical exam was performed once each week. Mortalities and moribund sacrifices were limited to three females. One high-dose and one low-dose female died on Days 63 and 77, respectively. The third female was sacrificed *in extremis* on Day 75. None of the deaths was treatment-related. Other than signs of dermal irritation, no treatment-related clinical signs of toxicity were present during the study. The incidence of gross dermal effects is presented in Table 2. Dermal

effects in females was more severe and extensive than in males. The treated skin of males showed only erythema and exfoliation in the high-dose group and, to a lesser extent, in the mid-dose group. Mid-dose females showed exfoliation and to a limited extent, excoriation and ulceration of the treated skin.

Table 2: Treated Skin: Incidence of Clinical Observations (Data summarized from Tables 1 and 2 of the study)

Observation	Sex	CON	LDT	MDT	HDT
Number of animals	♂/♀	15	15	15	15
Erythema	♂	0	0	0	3
	♀	0	0	0	7
Edema	♀	0	0	0	2
Exfoliation	♂	0	0	3	8
	♀	4	4	12	15
Excoriation	♀	0	0	1	6
Ulceration	♀	0	0	1	3

3. Dermal Irritation Results: Erythema and edema were graded at approximately three day intervals during the study using the Draize scoring method. The dermal effects in females were more severe than in males and lasted a greater number of days (Table 3). For females, erythema and edema were present through study Days 33 and 26, respectively. In males, no significant dermal effects were noted after study Day 8.

4. Body Weight and Body Weight Gains: Animals were weighed at the start of the study, weekly, thereafter, and at terminal sacrifice. No treatment-related effects were noted. The mean body weights and body weight gains for treated animals were comparable to controls throughout the study.

5. Food Consumption: Food consumption was measured at weekly intervals. Compared to controls, no significant differences in food consumption were noted in any of the treated groups at any time during the study.

6. Ophthalmological Examinations: Examinations were performed on all animals before the start of the study and before terminal sacrifice. A high incidence of mild corneal mineralization was present in all animals. Since these lesions were observed at both the prestudy and terminal sacrifice examinations, they were not treatment-related.

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Table 3: Summary of Erythema and Edema Draize Scores[a] (Data summarized from text table, page 14, of study)

Study Day	Erythema			Edema		
	LDT	MDT	HDT	LDT	MDT	HDT
Males						
5	0	2(1.5)	5(1.6)	0	1(1)	1(1)
8	0	1(2)	2(1)	0	1(1)	0
12	0	0	0	0	0	0
15	0	0	0	0	0	0
19	0	0	0	0	0	0
22	0	0	0	0	0	0
Females						
5	0	4(1.2)	13(2.5)	0	2(1)	9(1.6)
8	0	1(1)	8(1)	0	0	3(1)
12	0	0	11(1.3)	0	0	3(1)
15	1(2)	0	3(1)	0	0	0
19	0	0	6(1.8)	0	0	5(1)
22	0	0	5(1.4)	0	0	2(1)
26	0	0	2(1)	0	0	1(1)
29	0	0	0	0	0	0
33	0	1(1)	3(1)	0	0	0
36	0	0	0	0	0	0

[a] Number of animals with scores greater than 0, numbers in parentheses are the mean scores for animals with scores greater than 0.

7. Clinical Pathology: At terminal sacrifice, serum chemistry and hematological analyses were performed on fasted animals using blood collected from retroorbital sinus. Analyses were performed on all high-dose animals and ten animals per sex, randomly selected from each of the treatment groups.

a. Serum chemistry parameters: The following serum chemistry parameters listed below were evaluated. No treatment-related effects were noted in any of the parameters.

Electrolytes
- Calcium (Ca)
- Chloride (Cl)
- Sodium (Na)
- Phosphorous (P_i)
- Potassium (K)

Enzymes
- γ-Glutamyl transpeptidase (GGT)
- Alkaline phosphatase (ALK)
- Aspartate aminotransferase (SGOT/AST)
- Alanine aminotransferase (SGPT/ALT)

Other
- Glucose (GLU)
- Blood creatinine (CREAT)
- Blood urea nitrogen (BUN)
- Phospholipid (PL)
- Total bilirubin (TBIL)
- Direct bilirubin (DBIL)
- Indirect bilirubin (IBIL)
- Protein, total (PROT)
- Triglycerides (TG)
- Albumin (ALB)
- Globulins (GLOB)
- A/G ratio (A/G)

b. Hematology parameters: The following hematology parameters listed below were evaluated. No treatment-related effects were noted in any of the parameters.

Hematocrit (HCT)	Reticulocyte count (RC)
Hemoglobin (HGB)	Leukocyte differential count (LDC)
Leukocyte count (WBC)	Mean corpuscular HGB (MCH)
Erythrocyte count (RBC)	Mean corpuscular HGB conc. (MCHC)
Platelet count (PLAT)	Mean corpuscular volume (MCV)

8. Sacrifice and Pathology: Detailed pathological examination was performed on animals in the control and treatment groups. The tissues listed below were collected at necropsy and saved in 10% neutral buffered formalin. Histological examinations were performed on tissues (marked X or XX) from the control and high-dose animals; for the low and intermediate dose groups, the organs marked XX were examined. Selected tissues (CAPITAL LETTERS) were weighed before being fixed.

Digestive system	Cardiovas./Hematol	Neurologic
X Pancreas	X Aorta	X BRAIN
X Salivary glands	X HEART	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	X Pituitary
X Duodenum	X SPLEEN	X Eyes
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	X ADRENALS
X Cecum	XX KIDNEYS	X Lacrimal gland
X Colon	X Urinary bladder	X Mammary gland
X Rectum	X TESTES	X Parathyroids
XX LIVER	X Epididymides	X Thyroids
Respiratory	X Prostate	Other
X Trachea	X Cervix	XX Gross lesions
XX Lungs	X Vagina	XX Skin (treated and untreated)
	X OVARIES	X Skeletal muscle
	X Uterus	X Bone

a. Organ weights: Absolute and organ weights relative to both brain weight and body weight were measured. No significant, treatment-related findings were noted.

b. Gross pathology: Gross pathological examinations were performed on animals found dead or sacrificed in moribund condition during the study and surviving animals at terminal sacrifice. No treatment-related gross pathological changes were evident in any of the males. Gross observations in females was limited to an increased incidence of excoriation and exfoliation in treated skin. Of the 14 high-dose females, 5 showed excoriation and 7 showed exfoliation. At the mid-dose level, excoriation was present in only one animal.

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c. Microscopic pathology: Significant, treatment-related histopathological changes were noted only in treated skin; no treatment-related systemic lesions were present in any of the animals. Treatment-related dermal effects were limited to treated skin of mid-dose females and high-dose males and females (Table 4). The only significant finding in the high-dose males was an increased incidence of hyperkeratosis, which was graded as minimal to mild. In addition to hyperkeratosis, high-dose females showed minimal to moderate acanthosis, epidermitis and dermatitis and minimal to mild ulceration and vacuolar degeneration of the epidermis. The incidence of dermatitis was significantly increased in the mid-dose females.

Only two neoplastic lesions were noted, one in a low-dose female (lymphosarcoma) and the other in a high-dose male (adrenal adenoma). These lesions were considered to be incidental and not treatment-related effects.

Table 4: Treated Skin: Incidence of Histopathological Lesions (Dat summarized from Appendix 2, Tables 4, 5 7, and 8 of the study)

Observation	CON	LDT	MDT	HDT
Males				
Number examined	15	15	15	15
Hyperkeratosis	5 (3,2,0,0)[a]	1 (1,0,0,0)	2 (2,0,0,0)	10 (7,3,0,0)
Females				
Number examined	15	14	14	14
Hyperkeratosis	0	2 (2,0,0,0)	3 (3,0,0,0)	12** (4,8,0,0)
Acanthosis	0	9** (8,1,0,0)	4 (4,0,0,0)	6* (2,2,2,0)
Epidermitis	0	1 (1,0,0,0)	2 (2,0,0,0)	8** (2,3,2,1)
Dermatitis	0	0	6* (2,4,0,0)	10** (6,2,2,0)
Ulceration	0	0	0	2 (1,1,0,0)
Vacuolar degeneration, epidermis	0	0	0	3 (0,1,1,1)

[a] Incidence of severity of lesions (minimal, mild, moderate, marked)
* $p \leq 0.05$, ** $p \leq 0.01$

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D. DISCUSSION: Male and female Sprague-Dawley rats received repeated dermal dermal dosing of test compound at 0, 2, 6, or 12 mg/kg/day for 6 hours/day, 5 days/week for 13 weeks. No treatment-related effects were noted in mortality, body weight gain, food consumption, clinical pathology, or absolute and relative organ weights. No systemic toxicity was observed on gross or microscopic examinations. The only toxic effects were limited to treated skin of mid-dose females and high-dose males and females. The incidence and severity of the dermal effects was higher in females. Gross dermal lesions consisted of erythema, edema, exfoliation, excoriation and ulceration. The findings were confirmed by histopathological examination, where increased incidence of hyperkeratosis, acanthosis, epidermitis, dermatitis and ulceration were noted.

From the results of this study, NOELs and LOELs for systemic and dermal toxicity are as follows:

Toxicity		NOEL	LOEL
Systemic		> 12 mg/kg/day (HDT)	
Dermal	Males	6 mg/kg/day	12 mg/kg/day
	Females	2 mg/kg/day	6 mg/kg/day

The dermal LOEL is based is based increased incidence of histopathological lesions (hyperkeratosis, males and females and acanthosis, epidermitis, dermatitis and ulceration in females).

Core Classification: Minimum. This study satisfies guideline requirements (§82-3) for a 90-day dermal toxicity study in rats and is acceptable for regulatory purposes. Although systemic toxicity was not noted in this study, a systemic LOEL was established in a 90-day oral study in rats (MRID No.: 409663-02).

Reviewed by: Robert F. Fricke, Ph.D. Robt F. Fricke 23 Nov 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/22/93
Section IV, Tox. Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE:	Chronic Oral - Dogs [§83-1(b)]
DP BARCODES:	D188679, D189620
SUBMISSION NO.:	S436173
P.C. CODE:	069149
CASWELL NO.:	331A
MRID NO.:	419704-01
TEST MATERIAL:	BARDAC 2280
SYNONYMS:	Didecyldimethylammoniumchloride
STUDY NUMBER:	2545-102
SPONSOR:	Lonza, Inc., Fair Lawn, NJ
TESTING FACILITY:	Hazleton Washington, Inc., Vienna, VA
TITLE OF REPORT:	Chronic oral toxicity study of didecyldimethylammoniumchloride in dogs
AUTHOR:	G.E. Schulze
REPORT ISSUED:	26 July 1991

CONCLUSIONS: In a chronic, 1-year oral toxicity study, male and female beagle dogs were given test compound at dosages of 0, 3, 10, or 20/30 mg/kg body weight/day (Dosing at 30 mg/kg/day was not tolerated well and was discontinued on Day 31; dosing was resumed on Day 36 at 20 mg/kg/day). No treatment-related deaths occurred during the study. The treatment-related clinical signs (soft/mucoid feces, emesis) were observed frequently in high-dose animals. Hematology or urinalysis results were normal. Total cholesterol levels were significantly decreased in the high-dose females. Gross and histopathological findings did not reveal any treatment-related effects.

	NOEL	LOEL
Males and females	10 mg/kg/day	20 mg/kg/day (HDT)

The LOEL is based on increased incidence of clinical observations (emesis and soft/mucoid feces) in males and females and decreased total cholesterol levels in females.

CORE CLASSIFICATION: Guideline. This study satisfies guideline requirements [§83-1(b)] for a chronic oral study in dogs and is acceptable for regulatory purposes.

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A. MATERIALS

1. Test Compound: BARDAC 2280 Description: yellow liquid Batch #: B-1889 Purity: 80.8% [a.i.] Contaminants: not given

2. Test animals: Species: Dog Strain: Beagle Age: 8.5 - 9.5 months Weight (kg): 7.8 - 13.5 (males), 6.8 - 9.7 (females) Source: Hazleton Research Products, Inc., Cumberland, VA Housing: Individually in elevated cages Feed: Purina Certified Canine Diet #5007 Water: Tap water, *ad libitum* Environment: Temperature, 59 - 88°F; Humidity, 11 - 96%; Light cycle, 12 hr light/12 hr dark

B. METHODS

1. Animal Assignments: Animals were assigned randomly to main study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group		Dosage[a] (mg/kg/day)	Animals/Group Male	Female
Control	(CON)	0	4	4
Low	(LDT)	3	4	4
Mid	(MDT)	10	4	4
High	(HDT)	30/20[b]	4	4

a Dosage adjusted for percent purity of active ingredient

b Because of severe toxicity, dosing at 30 mg/kg/day dose was discontinued on Day 31; on Day 36 dosing was resumed at 20 mg/kg/day.

2. Dose Preparation: A weighed amount test compound was thoroughly mixed with basal diet to form a premix, which was refrigerated until used. An appropriate amount of premix was mixed with distilled water (Polar Distilled Water) to form the dosing slurries. The slurries were prepared fresh daily. The slurries were administered in two divided doses of 10 ml/kg/dose. Control animals received an appropriate amount of basal diet/water slurry.

3. Statistical Evaluations: Levene's test was used to evaluate homogeneity of variances. Homogeneous data were initially analyzed using a one-way analysis of variance (ANOVA). If the ANOVA result was significant (F-test), pair-wise comparisons were carried out using Dunnett's test. Heterogeneous data were transformed ($\log_{10}X$, X^2, $\sqrt{X}$, $1/X$, arcsine X, rank) and reevaluated for homogeneity. If transformed data was found to homogeneous, ANOVA and pair-wise comparisons were carried out.

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C. REGULATORY COMPLIANCE

1. Quality assurance was documented by signed and dated GLP and quality assurance statements.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS

1. Analytical Chemistry: The prepared diets were analyzed for stability, homogeneity and concentration. Test diets were stable for at least 14 days when refrigerated. Analysis of diet/water slurries, taken from the top, middle and bottom, indicated that the test compound was homogeneously distributed (relative standard deviations, 0.28 to 1.96%) and within 87.0 and 99.4% of nominal concentrations. The concentration of test compound was below the target on Day 1 (low-dose) and Day 2 (low- and middle-dose). Adjustments were made and for the remainder of the study, the concentration of test compound were all within ±10% of the target concentrations (91.0 to 103%, LDT; 91.3 to 106%, MDT; 93.8 to 110%, HDT).

2. Observations: Animals were inspected twice daily for signs of toxicity, mortality and moribundity. Detailed examinations were performed weekly.

a. Clinical observations: Table 2 summarizes the clinical observations noted during the study. Treatment-related effects consisted of soft and/or mucoid feces and emesis, described as frothy, containing food or compound-like material, or a combination of all three. Soft and/or mucoid feces was observed at least once in all of the middle- and high-dose animals, however, the frequency was greatest in the high-dose animals. Similarly, emesis, was observed more frequently in the high-dose animals, particularly during the first four weeks of the study when the animals were dosed at 30 mg/kg/day.

b. Mortality: An accidental death, attributed to gavage error, occurred in one low-dose male. All other animals survived until the scheduled sacrifice.

3. Body Weight and Body Weight Gain: Body weights were measured at the start of the study, at weekly intervals through Week 14, and every other week, thereafter. During the first four weeks of the study, the high-dose animals (dosed at 30 mg/kg/day) all experienced significant weight loss (Table 3).

Table 2: Incidence of Clinical Observations (Data summarized from Table 1 and Appendix 2 of the study)

Observation	Sex	CON	LDT	MDT	HDT
Found dead	♂	0	1	0	0
	♀	0	0	0	0
Soft and/or mucoid feces	♂	3* (1,2,3)	2 (2,2)	4 (1,2,3,5)	4 (6,14,20,24)
	♀	2 (1,9)	1 (1)	4 (1,1,7,17)	4 (2,4,8,27)
Emesis	♂	1 (1)	2 (2,3)	4 (2,4,6,7)	4 (12,15,21,24)
	♀	2 (2,7)	3 (1,2,1)	2 (1,8)	4 (2,6,13,23)

* Values in parenthesis are the number of days the observation was noted in each animal. For example, (1,4,7) denotes that three animals showed an effect 1, 4, and 7 times during the study.

Table 3: Mean Body Weight Gains (Data summarized from Table 3 of the study)

Sex	Week	CON	LDT	MDT	HDT
Male	0-4	0.3	0.3	0.3	-1.3*
Female	0-4	0.2	0.2	0.3	-0.3*

* $p \leq 0.05$

4. Food Consumption: Food consumption was measured at the at weekly intervals through Week 14 and every other week, thereafter. No treatment-related effects were noted in food consumption during the course of the study. At Week 1, the low- and high-dose females had food consumption values significantly less than that of the control. However, since the middle-dose animals showed no effect, the differences noted did not appear to be compound-related.

5. Ophthalmological examinations: Examinations were performed during the prestudy acclimation period and again before terminal sacrifice. No treatment-related eye lesions were observed.

6. Clinical Pathology: Clinical chemistry, hematology and urinalysis were performed before the start of the study to establish baseline values and at 13, 26, and 52 weeks of treatment.

a. Hematology: The following hematology parameters were examined:

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Cell morphology	Hematocrit (HCT)
Leukocyte count (WBC)	Corrected leukocyte count
Erythrocyte count (RBC)	Hemoglobin (HGB)
Leukocyte differential	Mean corpuscular HGB (MCH)
Platelet count	Mean corpuscular HGB conc. (MCHC)
Reticulocyte count	Mean cell volume (MCV)

Results: Although significant differences were noted in some hematology parameters, the magnitude of the responses was slight and judged not to be biologically important.

b. Clinical Chemistry: The following clinical chemistry parameters were examined:

Electrolytes
- Calcium (Ca)
- Chloride (Cl)
- Sodium (Na)
- Phosphorous (P_i)
- Potassium (K)

Enzymes
- γ-Glutamyl transpeptidase (GGT)
- Creatine kinase (CK)
- Alanine aminotransferase (SGPT/ALT)
- Aspartate aminotransferase (SGOT/AST)

Other
- Glucose (GLU)
- Blood creatinine (CREAT)
- Blood urea nitrogen (BUN)
- Total bilirubin (TBIL)
- Protein, total (PROT)
- Albumin (ALB)
- Total cholesterol (CHOL)
- Globulin (GLOB)

Results: Statistically significant differences were noted in high-dose males (total protein, albumin and creatine kinase) and females (sodium), however, the values were within historical control ranges (reviewer's reference, historical control values were not included with the study) and therefore of questionable biological significance. In the high-dose females, total cholesterol levels were lower than the control values at each of the time points, with a significanct difference occurring at Week 13 (Table 4).

Table 4: Total Cholesterol Levels (mg/dl) for Female Dogs (Data summarized from Table 7 of the study)

Week	CON	LDT	MDT	HDT
-2	143	163	157	158
13	169	189	127	102*
26	142	156	141	112
52	153	248	167	136

* $p \leq 0.05$

c. Urinalysis: The following urinalysis parameters were examined:

Appearance	Glucose
Specific gravity	Ketone Bodies
Protein	Bile Pigments
Volume	Urobilirubin
Sediment	Total Bilirubin
pH	Occult Blood
Fecal flotation	Reducing substances
Nitrites	

Results: No treatment-related effects were noted in any of the animals.

8. Sacrifice and Pathology: Detailed pathological examination was performed on male and female animals in the control and treatment groups. The tissues listed below were fixed in 10% neutral buffered formalin and examined histologically. Selected organs (CAPITAL LETTERS) were also weighed.

Digestive system
- Pancreas
- Salivary glands
- Esophagus
- Stomach
- Duodenum
- Jejunum
- Ileum
- Cecum
- Colon
- Rectum
- LIVER
- Gallbladder

Respiratory
- Lungs
- Trachea

Cardiovas./Hematol
- Aorta
- HEART
- Bone marrow
- Lymph nodes
- SPLEEN
- Thymus

Urogenital
- KIDNEYS
- Urinary bladder
- TESTES/Epididymides
- OVARIE
- Prostate
- Uterus

Neurologic
- BRAIN
- Periph. nerve
- Spinal cord
- PITUITARY
- Eyes + optic nerve

Glandular
- ADRENALS
- THYROIDS/Parathyroids
- Mammary gland

Other
- Gross lesions
- Skin
- Bone
- Skeletal muscle

a. Organ Weights: No treatment-related effects were noted in either the absolute or relative organ weights. All values were comparable to controls.

b. Gross Pathology: Gross examination of tissues taken at the terminal sacr[illegible] did not reveal any treatment-related abnorm[illegible]

c. Histopathology: His[illegible]cal examination at terminal sacrifice did n[illegible] any changes which could be attributable to [illegible]nt.

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E. DISCUSSION: In a chronic, 1-year oral toxicity study, male and female beagle dogs were gavaged with test compound at dosages of 0, 3, 10 or 20/30 mg/kg body weight/day. The high-dose (30 mg/kg/day exceeded the maximum tolerated dose, since after approximately four weeks of dosing, some animals experienced significant decreases in body weight and a high incidence of emesis and soft feces. Dosing at 30 mg/kg/day was discontinued on Day 31 and resumed on Day 36 at 20 mg/kg/day. This change did not appear to affect the outcome of the study.

With the exception of the accidental death of one low-dose male (attributed to gavage error), all animals survived to terminal sacrifice. The treatment-related clinical signs observed in the high-dose animals included a high incidence of soft/mucoid feces and emesis. These clinical signs persisted throughout the study. Although these clinical signs were noted in other treatment groups, the occurrence was sporadic.

Clinical pathology did not reveal any treatment-related effects in either hematology or urinalysis. Clinical chemistry effects were considered slight and not biologically significant. A possible treatment-related effect was a significant decrease in total cholesterol in the high-dose females at Week 13. Gross and histopathological findings did not reveal any treatment-related effects. No neoplastic lesions were noted.

	NOEL	LOEL
Males and females	10 mg/kg/day	20 mg/kg/day)HDT)

The LOEL is based on increased incidence of clinical observations (emesis and soft/mucoid feces) in males and females and decreased total cholesterol levels in females.

CORE CLASSIFICATION: Guideline. This study satisfies guideline requirements [§83-1(b)] for a chronic oral toxicity study in dogs and is acceptable for regulatory purposes.

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 23 Nov 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/23/93
Section IV, Tox. Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE:	Carcinogenicity - Mouse [§83-2(b)]
DP BARCODES:	D188679 D189620
SUBMISSIONS:	S436173
P.C. CODE:	069149
CASWELL NO.:	331A
MRID NO.:	418023-01
TEST MATERIAL:	BARDAC 2280
SYNONYMS:	Didecyldimethylammoniumchloride
STUDY NUMBER:	53-528
SPONSOR:	Lonza, Inc., Fair Lawn, NJ
TESTING FACILITY:	Bushy Run Research Center, Export, PA
TITLE OF REPORT:	Chronic dietary oncogenicity study with didecyldimethylammoniumchloride in mice
AUTHOR:	M.W. Gill, S.J. Hermansky and C.L. Wagner
REPORT ISSUED:	7 February 1991

CONCLUSIONS: In this carcinogenicity study, male and female mice were fed diets containing BARDAC 2280 at concentrations of 0, 100, 500, or 1000 ppm (mg/kg/day equivalents: 0, 15.0, 76.3, or 155.5 for males and 0, 18.6, 93.1, or 193.1 for females). No treatment-related effects were noted in the incidence of clinical signs, deaths, gross and histopathological observations. Hematological values were comparable among all study groups. Effects attributable to treatment included decreased mean body weights and body weight gains of high-dose males and females.

	NOEL	LOEL
Males and females	500 ppm (76.3 mg/kg/day)	1000 ppm (155.5 mg/kg/day)

The LOEL is based on decreased mean body weights and body weight gains.

CORE CLASSIFICATION: Minimum. This study satisfies guideline requirements [§83-2(b)] for a carcinogenicity study in mice and is acceptable for regulatory purposes.

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A. MATERIALS

1. Test Compound: BARDAC 2280 Description: yellow liquid Batch #: B-1889 Purity: 80.8% [a.i.] Contaminants: not given

2. Test animals: Species: Mouse Strain: CD-1 Age: 8 weeks Weight (g): 24.9 - 33.1 (males), 19.7 - 26.4 (females) Source: Charles River Breeding Laboratories, Inc, Portage, MI Housing: Individually in stainless steel cages with wire mesh floor Feed: Ground Purina Certified Rodent Chow /5002 Water: Tap water, *ad libitum* Environment: Temperature, 66 - 75°F; Humidity, 40 - 70%; Light cycle, 12 hr light/12 hr dark

B. METHODS

1. Animal Assignments: Animals were assigned randomly to main study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group		Dosage[a] (ppm)	Animals/Group Male	Female
Control 1	(CON1)	0	60	60
Low	(LDT)	100	60	60
Mid	(MDT)	500	60	60
High	(HDT)	1000	60	60
Control 2	(CON2)	0	60	60

[a] Dosage adjusted for percent purity of active ingredient

2. Justification for Dose Selection: Doses used in this study were based on dose range-finding studies (BBRC Report Numbers 51-507 and 51-561). These studies, however, were not submitted to the Agency for review.

3. Diet Preparation: A weighed amount test compound was thoroughly mixed with basal diet to form a concentrated premix. The premix was thoroughly mixed to ensure evaporation of ethanol. Diets were prepared by serial dilution of the premix or higher diet concentrations.

4. Statistical Evaluations: Parametric data were initially evaluated for homogeneity of variances using Levene's test. Homogeneous data were then analyzed using a one-way analysis of variance (ANOVA) and pooled variance t-tests. Data found to be heterogeneous were evaluated using ANOVA for unequal variances followed by separate variance t-tests. Parametric data were evaluated using either the Kruskal-Wallis test or the Mann-Whitney-modified Wilcoxon rank sum test. Frequency data were evaluated using the Fisher's exact test.

This study contained two control groups. For statistical purposes, each control group was treated separately.

C. REGULATORY COMPLIANCE

1. Quality assurance was documented by signed and dated GLP and quality assurance statements.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS

1. Analytical Chemistry: The prepared diets were analyzed for stability, homogeneity and concentration. The 100 and 1000 ppm test diets were stable for at least 21 days room temperature and were within 98.0 to 105% and 98.9 to 107.3% of nominal, respectively. For determination of homogeneity, samples were taken from the top, middle and bottom of the mixing bowl. The 100, 500 and 1000 ppm diets were within 96.0 to 107.0%, 102.0 to 109.4% and 104.5 to 111.7% of nominal, respectively. Verification of concentration was performed weekly for the first month and at monthly intervals, thereafter. Throughout the study, the concentrations of test compound were within 95.5 to 109.6% of nominal.

2. Observations: Animals were inspected twice daily for signs of toxicity, mortality and moribundity. Detailed clinical examinations were performed weekly.

a. Clinical observations: No clinical signs attributable to treatment were observed during the study. The incidence of palpable masses in males and females was comparable among all of the dose groups.

b. Mortality: A summary of animals fate data is presented in Table 2. The percent mortalities (excluding accidental deaths) and mean survival times were comparable among all of the study groups.

3. Body Weight and Body Weight Gain: Body weights were measured at the start of the study, at weekly intervals through Week 14, and every other week, thereafter.

a. Body weights: Mean body weights for selected time points are summarized in Table 3, below. Consistent, significant effects on body weights were limited to animals in the high-dose groups. Other significant findings were present in the low- and mid-dose groups, but were sporadic and not considered treatment-related.

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Table 2: Summary of Mortality (Data summarized from Table 1 of study)

Fate of animals	Sex	0 ppm[1]	300 ppm	750 ppm	1500 ppm	0 ppm[2]
Animals on Study	♂,♀	60	60	60	60	60
Found Dead	♂	7	9	8	8	14
	♀	14	9	10	7	8
Moribund Sacrifices	♂	6	3	6	5	5
	♀	2	3	5	5	2
Percent Mortality	♂	22	20	23	22	32
	♀	28	20	25	20	17
Accidental Deaths	♂	0	0	0	0	0
	♀	1	0	0	0	1
Terminal Sacrifice	♂	47	48	46	47	41
	♀	43	48	45	48	49
Mean Survival Time	♂	536	532	534	532	511
	♀	534	540	536	539	545

[1] Control 1
[2] Control 2

Table 3: Mean Body Weights (Data summarized from Tables 8 and 10 of study)

		Mean Body Weight (g)				
Sex	Week	0 ppm[1]	300 ppm	750 ppm	1500 ppm	0 ppm[2]
Males	0	29.1	29.1	28.9	28.5	28.5
	1	30.4	30.0	30.2[a]	29.5[a]	29.4[b]
	2	31.2	31.2	31.2	30.0[b]	30.6
	4	33.2	33.6[a]	33.1	32.0[b]	32.5
	8	35.6	35.6	35.1	34.7	35.0
	12	37.0	37.2	36.4	35.9	36.6
	20	39.0	39.1	38.0	37.5[b]	38.4
	36	41.3	41.8	40.0	38.9[bd]	40.9
	52	42.1	43.1	40.9	39.9[bd]	42.0
	78	42.1	43.2	41.1	40.0[bd]	42.5
Females	0	23.0	23.0	23.0	22.9	22.8
	1	23.8	23.6	23.5	23.5	23.7
	2	24.9	24.7	24.4	23.6[bd]	24.9
	4	26.5	26.1	25.8[a]	25.3[bd]	26.1
	8	28.3	28.0	27.9	27.2[b]	27.9
	12	30.2	30.0	29.2[a]	28.5[bd]	29.6
	20	31.7	31.8	31.0	29.7[bd]	31.2
	36	34.9	34.7	33.5	31.7[bd]	34.2
	52	36.3	36.3	34.8[a]	33.0[bd]	36.0
	78	37.4	36.6	36.0	33.9[bd]	37.4

[1] CON1
[2] CON2
[a] Significant difference from CON1, $p \leq 0.05$
[b] Significant difference from CON1, $p \leq 0.01$
[c] Significant difference from CON2, $p \leq 0.05$
[d] Significant difference from CON2, $p \leq 0.01$

b. Body weight gains: Body weight gains for selected intervals are summarized in Table 4. For high-dose males, body weight gains were lower than control values for the entire study. Differences were statistically significant up to Week 4 and from Week 16 to the end of the study. Body weight gains for high-dose females were significantly lower than controls throughout the entire study. Body weight gains for mid-dose females were lower than controls with occasional significant differences. Although significant findings were noted in mid-dose males, the differences were, in general, only significantly different from only one, but not both controls. For mid-dose females, significant findings were, in general, present through Week 5 of the study. Thereafter, only occasional significant findings were observed.

In the absence of any treatment-related effects in other parameters (food consumption, hematology, organ weights, gross and histopathology) at the mid-dose(500 ppm), the occasional statistical differences observed in body weight gains were not considered to be either biologically significant or treatment-related.

Table 4: Mean Body Weight Gains (Data summarized from Tables 9 and 11 of study)

		Mean Body Weight Gains (g)				
Sex	Week	0 ppm[1]	300 ppm	750 ppm	1500 ppm	0 ppm[2]
Males	0 - 1	1.3	0.9[a]	1.3	1.0[a]	0.9[a]
	0 - 2	2.1	2.1	2.3	1.8[a,b]	2.1
	0 - 4	4.1	4.5[c]	4.2	3.5[a,b]	4.0
	0 - 8	6.5	6.5	6.2	6.2	6.5
	0 - 12	7.5	8.1	7.5	7.4	8.1
	0 - 20	9.9	10.0	9.1[c]	9.0[a,b]	9.9
	0 - 36	12.2	12.7	11.1[a,b]	10.4[a,b]	12.4
	0 - 52	13.0	14.0	12.0[c]	11.5[a,b]	13.7
	0 - 78	13.4	14.2	12.5[c]	11.7[a,b]	14.3
Females	0 - 1	0.8	0.6[c]	0.6[c]	0.6[c]	0.9
	0 - 2	1.8	1.7	1.4[a,b]	0.9[a,b]	2.1
	0 - 4	3.4	3.2	2.8[a,b]	2.4[a,b]	3.3
	0 - 8	5.3	5.1	5.0	4.3[a,b]	5.1
	0 - 12	7.2	7.0	6.3[b]	5.6[a,b]	6.8
	0 - 20	8.7	8.8	8.1	6.8[a,b]	8.4
	0 - 36	11.8	11.7	10.6[c]	8.8[a,b]	11.4
	0 - 52	13.3	13.3	11.9[a,b]	10.1[a,b]	13.3
	0 - 78	14.5	13.8	13.1[c]	11.2[a,b]	14.5

[1] CON1
[2] CON2
[a] Significant difference from CON1 ($p \leq 0.05$)
[b] Significant difference from CON1 ($p \leq 0.01$)
[c] Significant difference from CON2 ($p \leq 0.05$)
[d] Significant difference from CON2 ($p \leq 0.01$)

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4. Food Consumption: Food consumption was measured at weekly intervals through Week 14 and every other week, thereafter. Statistically significant differences in food consumption were noted in both males and females throughout the study (Table 5). Significant occurrences were, however, sporadic in nature and therefore, did not to appear to be a treatment-related effect.

Table 5: Food Consumption (Data summarized from Tables 4 and 5 of study)

Sex	Week	Food Consumption (g/animal/day) 0 ppm[1]	300 ppm	750 ppm	1500 ppm	0 ppm[2]
Male	0-1	6.0	6.2	6.3	6.7[M]	6.3
	1-2	5.9	5.9	5.9	5.5[M]	5.8
	5-6	6.0	6.0	6.4[M]	5.8	5.9
	11-12	5.9	5.9[a]	5.8	5.7[a]	5.7[a]
	31-32	5.6	5.8[a]	5.6	5.5[a]	5.3
	33-34	5.9	6.0	5.7[a]	5.8	5.9
	35-36	5.5	5.7	6.5	5.3[a]	5.5
	45-48	5.8	5.8[a]	6.0[a]	6.2[b]	6.3[b]
	55-56	5.4	5.6[a]	5.5[a]	5.5[a]	5.7[b]
	57-58	5.9	5.9	5.8[d]	5.7[a]	6.2
	61-62	5.2	5.3[d]	5.3[c]	5.3[d]	5.5[b]
	63-64	5.4	5.5	5.3[c]	5.2[M]	5.5
	65-66	5.6	5.4[a]	5.2[M]	5.0[M]	5.7
	71-72	5.4	5.4	5.2[c]	5.1[M]	5.5
	77-78	5.4	5.7	5.1[M]	5.0[M]	5.3
Female	0-1	5.9	6.3	5.8	6.9[M]	5.9
	1-2	5.3	5.2[d]	5.2[d]	5.6	5.9[b]
	2-3	6.3	6.6	6.1[c]	6.0[d]	6.6
	5-6	5.8	6.2[a]	5.9	5.6[d]	6.1
	9-10	6.1	6.5	6.3	5.9[d]	6.3
	15-16	6.4	6.8	6.2	5.9[a]	6.2
	23-24	6.3	6.1[d]	6.7	6.2[a]	6.7
	27-28	6.1	5.7[d]	5.8[d]	6.4	6.6[a]
	43-44	5.5	5.5	5.8[a]	5.9[b]	5.8[a]
	65-66	5.6	5.7	5.1[M]	4.9[M]	5.6

[1] CON1
[2] CON2
[a] Significant difference from CON1 ($p \leq 0.05$)
[b] Significant difference from CON1 ($p \leq 0.01$)
[c] Significant difference from CON2 ($p \leq 0.05$)
[d] Significant difference from CON2 ($p \leq 0.01$)

5. Achieved Compound Intake: The overall mean values for achieved compound intake are summarized in Table 6.

Table 6: Compound Intake (mg/kg/day) for Weeks 0 to 78 (Data summarized from Tables 6 and 7 of study)

Sex	100 ppm	500 ppm	1000 ppm
Male	15.0	76.3	155.5
Female	18.6	93.1	193.1

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6. Hematology: Hematology measurements were performed on high dose and both controls groups (10 animals/sex/group) during Weeks 52 and 78 of the study; differential leukocyte counts were performed at 12 and 18 months. The following parameters were evaluated:

Hematocrit (HCT)	Leukocyte differential count (LDC)
Hemoglobin (HGB)	Mean corpuscular volume (MCV)
Leukocyte count (WBC)	Mean corpuscular HGB (MCH)
Erythrocyte count (RBC)	Mean corpuscular HGB conc. (MCHC)
Platelet count (PLAT)	

No treatment-related effects were noted in any of the hematology parameters.

8. Sacrifice and Pathology: Detailed pathological examination was performed on male and female animals in the control and treatment groups. No interim sacrifices (as required by guidelines) were performed; all surviving animals were sacrificed at 79 weeks. The tissues listed below were fixed in 10% neutral buffered formalin. Tissues from the two control groups and the high-dose group were examined histologically, while the lungs, livers, kidneys and gross lesions were examined for all dose groups. Selected organs (CAPITAL LETTERS) were also weighed. Terminal body weights were also recorded.

Digestive system
- Pancreas
- Salivary gland
- Esophagus
- Stomach
- Duodenum
- Jejunum
- Ileum
- Cecum
- Colon
- Rectum
- LIVER
- Gall Bladder

Respiratory
- Lungs
- Trachea

Cardiovas./Hematol
- Aorta
- HEART
- Bone marrow
- Lymph nodes
- SPLEEN
- Thymus

Urogenital
- KIDNEYS
- Urinary bladder
- TESTES
- Ovaries
- Prostate
- Uterus
- Epididymis
- Vagina
- Seminal vesicles

Neurologic
- BRAIN with stem
- Periph. nerve
- Spinal cord
- Pituitary
- Eyes

Glandular
- Adrenals
- Thyroids
- Mammary gland

Other
- Gross lesions
- Skin
- Bone
- Skeletal muscle

a. Organ Weights: At terminal sacrifice, selected organs were weighed; significant findings are summarized in Table 7, below. Significant difference in absolute and relative organ weights were generally limited to high-dose animals. These differences appeared, however, to be a reflection of the significant decrease in terminal body weights, rather than a treatment-related effect.

Table 7: Terminal Body Weights and Absolute and Relative Organ Weights (Data summarized from Tables 11, 12, 13, 14 and 15 of study)

Observation	Sex	0 ppm	100 ppm	500 ppm	1000 ppm	0 ppm
Terminal Body Weights (g)						
	♂	41.8	43.6	41.1	39.7[a,d]	42.5
	♀	37.4	35.9	35.5[a]	34.0[a,d]	36.6
Absolute Organ Weights (g)						
Liver	♂	2.29	2.54[a]	2.38	2.14[a,d]	2.39
Organ Weights Relative to Terminal Body Weight (%)						
Brain	♂	1.22	1.18	1.24	1.28[a,d]	1.21
	♀	1.39	1.43	1.44	1.50[a,d]	1.42
Kidneys	♀	1.43	1.51	1.45	1.56[a,d]	1.47
Organ Weights Relative to Brain Weight (%)						
Liver	♂	455	504	478	425[a,d]	470

[a] Significant difference from CON1, $p \leq 0.05$
[b] Significant difference from CON1, $p \leq 0.01$
[c] Significant difference from CON2, $p \leq 0.05$
[d] Significant difference from CON2, $p \leq 0.01$

b. Gross Pathology: Examination of tissues taken at th terminal sacrifice did not reveal any treatment-related gross pathological lesions.

c. Microscopic Pathology: Histopathological examinatio terminal sacrifice did not reveal any treatment-related changes in the incidences of non-neoplastic or neoplastic lesions in either sex at any dose level. Non-neoplastic neoplastic lesions commonly seen in aging/aged mice occur in a similar extent in both treated and control mice. A summary of all neoplastic lesions is attached (Appendix

E. DISCUSSION: In this carcinogenicity study, male and female mi were fed diets containing BARDAC 2280 at concentrations of 0, 100, 500, or 1000 ppm (mg/kg/day equivalents: 0, 15.0, 76.3, or 155.5 males and 0, 18.6, 93.1, or 193.1 for females). No treatment-relat effects were noted in the incidence of clinical signs, deaths, gro and histopathological observations. Hematological values were comparable among all study groups. Effects attributable to treatm included decreased mean body weights and body weight gains of high-dose males and females.

In a parallel study (MRID No.: 419651-01), male and female rats w fed diets containing BARDAC 2280 at 0, 300, 750 or 1500 ppm (mg/kg/ equivalents: 0, 13, 32, or 64 for males and 0, 16, 41, or 83 for females) for two years. High-dose animals showed significant, but slight (< 10%) decreases in mean body weight during the study. Treatment related effects consisted of increased incidence of sinusoidal blood, hemosiderosis and histiocytosis in the mesenteri lymph nodes of high dose animals. The incidence of neoplastic les in treated animals was comparable to controls. BARDAC 2280 was not carcinogenic in male or female rats.

F. CONCLUSIONS

Adequacy of the high dose tested to assess carcinogenicity: Although the highest dose tested (1000 ppm) did not induce any treatment-related effects on survival, hematology, clinical signs, organ weights, gross pathology or histopathology, it produced consistent, statistically significant decreases (≥ 10%) in mean body weight and mean body weight gain in both sexes throughout the study. Therefore, it is concluded that this dose was adequate to assess the carcinogenic potential of BARDAC 2280.

Under the conditions of the study, the following NOEL and LOEL are established for chronic toxicity:

NOEL =	500 ppm	76.3 mg/kg/day, males 93.1 mg/kg/day, females
LOEL =	1000 ppm	155.5 mg/kg/day, males 193.1 mg/kg/day, females

LOEL was based on decreased mean body weight and body weight gain

At the dose levels tested, BARDAC 2280 was not carcinogenic in male or female mice.

CORE CLASSIFICATION: Minimum. This study satisfies guideline requirements [§83-2(b)] for a carcinogenicity study in mice and is acceptable for regulatory purposes.

G. DEFICIENCY: An interim sacrifice was not performed at 12 months as requested by the Subdivision F Guidelines. This deviation is not expected to affect the results of the study.

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APPENDIX 1: SUMMARY OF NEOPLASTIC LESIONS FOR ALL ANIMALS ON STUDY (Data taken from Appendix 2, Tables 14 and 18 of study)

Page_____ is not included in this copy.

Pages 94 through 108 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 document is not responsive to the request.

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Reviewed by: Robert F. Fricke, Ph.D. Robt F. Fricke 2/3/94
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/23/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Combined Chronic/oncogenicity - Rats (83-5(a))

DP BARCODES: D188679 D189620 **SUBMISSION:** S436173

P.C. CODE: 069149 **CASWELL NO.:** 331A

MRID NO.: 419651-01

TEST MATERIAL: BARDAC 2280

SYNONYMS: Didecyldimethylammoniumchloride

STUDY NUMBER: 53-566

SPONSOR: Lonza, Inc., 17-17 Route 208, Fair Lawn, NJ

TESTING FACILITY: Bushy Run Research Center, R.D. #4, Mellon Road, Export, PA

TITLE OF REPORT: Chronic dietary toxicity/oncogenicity study with decyldimethylammoniumchloride

AUTHOR: M.W. Gill, J.S. Chun and C.L. Wagner

REPORT ISSUED: 27 June 1991

CONCLUSIONS: Male and female rats were fed diets containing BARDAC 2280 at 0, 300, 750 or 1500 ppm (mg/kg/day equivalents: 0, 13, 32, or 64 for males and 0, 16, 41, or 83 for females) for two years. High-dose animals showed significant, but slight (< 10%) decreases in mean body weight during the study. Treatment related effects consisted of increased incidence of sinusoidal blood, hemosiderosis and histiocytosis in the mesenteric lymph nodes of high dose animals. The incidence of neoplastic lesions in treated animals was comparable to controls. BARDAC 2280 was not carcinogenic in male or female rats.

	NOEL	LOEL
Males & Females	750 ppm (MDT)	1500 ppm (HDT)

The LOEL is based on increased incidence on nonneoplastic lesions in the mesenteric lymph nodes (sinusoidal blood, hemosiderosis and histiocytosis).

CORE CLASSIFICATION: Minimum. This study satisfies guideline requirements [§83-5(a)] for a combined chronic toxicity/oncogenicity study in rats and is acceptable for regulatory purposes.

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A. MATERIALS:

1. Test compound: BARDAC 2280 Description: viscous, honey-colored liquid Batch #: B-1889 Purity: 80.8% [a.i.] Contaminants: not given

2. Test animals: Species: Rat Strain: Sprague-Dawley CD Age: 8 weeks Weight (g): 237.7 - 295.9 (males), 154.8 - 199.7 (females) Source: Charles River Breeding Laboratories, Inc., Portage, MI. Housing: Individually in suspended cages Feed: Purina Certified Rodent Chow #5002 Water: Tap water, *ad libitum* Environment: Temperature, 66 - 75 °F; Humidity, 40 - 70%; Light cycle, 12 hr light/12 hr dark; Air changes, at least eight per hour.

B. METHODS

1. Animal Assignments: Animals were assigned randomly to main study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Test Group		Dosage* (ppm)	Animals/Group Male	Female
Control 1	(CON1)	0	60	60
Low	(LDT)	300	60	60
Mid	(MDT)	750	60	60
High	(HDT)	1500	60	60
Control 2	(CON2)	0	60	60

* Dosage adjusted for percent purity of active ingredient

2. Justification for Dose Selection: Doses for this study were selected based on dose range-findings studies (BBRC Report Numbers 51-506 and 51-560). The 90-day study (51-506) was submitted and reviewed by the Agency (MRID No. 409663-02).

3. Diet Preparation: A weighed amount test compound was mixed with basal diet to form a concentrated premix, which was thoroughly mixed to ensure evaporation of ethanol. Diets were prepared by serial dilution of the premix for higher diet concentrations.

4. Statistical Evaluations: Parametric data were initially evaluated for homogeneity of variances using Levene's test. Homogeneous data were then analyzed using a one-way analysis of variance (ANOVA) and pooled variance t-tests. Data found to be heterogeneous were evaluated using ANOVA for unequal variances followed by separate variance t-tests. Parametric

data were evaluated using either the Kruskal-Wallis test or the Mann-Whitney-modified Wilcoxon rank sum test. Frequency data were evaluated using the Fisher's exact test.

This study contained two control groups. For statistical purposes, each control group was treated separately.

C. REGULATORY COMPLIANCE

1. Quality assurance was documented by signed and dated GLP and quality assurance statements.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS

1. Analytical Chemistry: The prepared diets were analyzed for stability, homogeneity and concentration. The 300 and 1500 ppm test diets were stable for at least 21 days at room temperature and were 99.9 to 108% and 97.6 to 105.8% of nominal respectively. For determination of homogeneity, samples were taken from the top, middle and bottom of the mixing bowl. The 300, 750 and 1500 ppm diets were within 98.3 to 107.0%, 102.1 to 106.1% and 95.1 to 104.3% of nominal, respectively. Verification of concentration was performed weekly for the first month and at monthly intervals, thereafter. Throughout the study, the concentrations of test compound were within 91.6 to 109.2% of nominal.

2. Observations: Animals were inspected twice daily for signs of toxicity, mortality and moribundity. Detailed clinical examinations, including palpable masses, were performed weekly.

a. Clinical observations: No clinical signs attributable to treatment were observed during the study. The incidence of palpable masses in males and females was comparable among all of the dose groups.

b. Mortality: A summary of animal fate data is presented in Table 2. The percent mortalities (excluding accidental deaths) and mean survival times were comparable among all of the study groups.

4. Body Weight and Body Weight Gain: Body weights were measured at the start of the study, at weekly intervals through Week 14, and every other week, thereafter.

Table 2: Summary of Mortality (Data summarized from Table 1 of study)

Fate of animals	Sex	Dietary Concentration (ppm) 0[a]	300	750	1500	0[b]
Found dead	♂	14	15	21	13	13
	♀	8	11	8	10	9
Moribund sacrifice	♂	7	18	12	13	15
	♀	22	13	12	12	21
Percent Mortality[c]	♂	35	55	43	47	47
	♀	50	40	33	37	50
Accidental deaths	♂	2	0	0	1	0
	♀	0	0	1	0	0
Terminal sacrifice	♂	37	27	27	33	32
	♀	30	36	39	38	30
Mean survival time (days)	♂	692	658	673	677	599
	♀	675	684	675	699	669

[a] Control 1
[b] Control 2
[c] Excludes accidental deaths

a. Body weights: Mean body weights for selected time intervals are summarized in Table 3. Consistent decreases in body weights were limited to animals in the high-dose groups. The mean body weights of high-dose males were lower than either control for the entire study. Statistically significant changes from both controls were noted from Weeks 4 to 8, Week 10, and Weeks 18 to 20; values were significantly lower than the first control on Weeks 22, 24, and 88. The mean body weights of high-dose females were lower than either control for the entire study. Statistically significant changes from both controls were limited to Weeks 5, 6, and 7. Significant differences form the first, but not second, control were noted on Weeks 4, 11, 13 to 50, 54, and 58 to 104. Overall, the changes in mean body weights for the high-dose animals were considered slight. For males, body weights were 0.4 to 6.5% lower than the first control and 2.0 to 4.1% lower than the second control. For most of the study, female body weights were 1.2 to 7.3% lower than the first control and 0.6 to 6.0% lower than the second control. Body weights for high-dose females at Week 104 were 11.4% lower than values for the first control. Other significant findings were present in the low- and mid-dose groups, but were sporadic in nature and not considered treatment-related.

Table 3: Mean Body Weights (Data summarized from Tables 5 and 7 of study)

Week	Mean Body Weights, g				
	0 ppm (CON1)	300 ppm	750 ppm	1500 ppm	0 ppm (CON2)
Males					
0	269	267 (-0.6, 0.5)*	268 (0.5, 1.0)	266 (-0.4, 0.1)	266
1	316	315 (-0.5, 0.1)	315 (0.2, 0.3)	308 (-2.1, -2.0)	314
2	352	352 (0.2, 0.2)	352 (0.1, 0.2)	344 (-2.3, -2.2)	352
4	405	405 (0.0, 0.4)	406 (0.3, 0.7)	392[d] (-3.2, -2.9)	403
8	486	482 (-0.8, 0.1)	484 (0.4, 0.5)	467[d] (-3.0, -2.9)	481
12	531	526 (-0.8, 0.5)	530 (0.6, 1.)	509 (-3.2, -2.8)	524
16	561	561 (-0.1, 0.8)	560 (-0.2, 0.5)	540 (-3.8, -3.1)	557
24	603	597 (-0.9, 1.6)	596 (-0.1, 1.5)	572[c] (-4.3, -2.7)	587
32	630	627 (-0.5, 0.3)	630 (0.5, 0.8)	603 (-3.8, -3.6)	625
40	664	663 (-0.1, 0.5)	666 (0.5, 1.1)	640 (-3.4, -2.9)	659
56	710	705 (-0.7, -0.1)	710 (0.7, 0.6)	680 (-3.6, -3.8)	706
72	738	729 (-1.2, 0.2)	733 (0.6, 0.8)	702 (-3.7, -3.5)	727
88	763	740 (-2.9, 2.6)	736 (-0.6, 2.0)	692[c] (-6.5, -4.1)	722
104	701	681 (-2.8, -1.3)	699 (2.6, 1.3)	677 (-0.6, -1.9)	690
Females					
0	179	179 (0.2, 1.5)	177 (-0.8, 0.7)	177 (-1.2, 0.3)	176
1	198	199 (0.5, 0.6)	199 (0.0, 0.6)	197 (-1.2, -0.6)	198
2	216	216 (0.0, 1.1)	216 (-0.2, 0.9)	212 (-1.9, -0.8)	214
4	243	244 (0.3, 1.5)	243 (-0.4, 1.2)	235[b] (-3.5, -2.0)	240
8	274	274 (0.2, 1.1)	274 (-0.3, 0.8)	265 (-3.4, -2.2)	271
12	290	291 (0.3, 1.6)	290 (-0.3, 1.2)	280 (-3.9, -2.4)	286
16	307	306 (-0.5, 1.2)	307 (0.5, 1.7)	293[c] (-4.2, -3.1)	302
24	328	324 (-1.1, 1.1)	329 (1.4, 2.5)	310[c] (-4.3, -3.3)	321
32	356	348 (-2.3, 1.0)	356 (2.4, 3.4)	334[c] (-3.9, -2.9)	344
40	384	377 (-2.0, 1.9)	384 (1.8, 3.7)	356[c] (-5.6, -3.9)	370
56	423	418 (-1.2, 1.9)	429 (2.5, 4.4)	396 (-5.3, -3.5)	411
72	455	453 (-0.5, 3.4)	467 (3.2, 6.7)	425[b] (-6.2, -3.0)	438
88	499	488 (-2.2, 1.4)	519[c] (6.2, 7.7)	453[b] (-7.3, -6.0)	482
104	492	480 (-2.4, 7.2)	501 (4.2, 11.7)	425 (-11.4, -5.1)	448

* Values in parentheses represent percent of control (% of CON1, % of CON2)
[b] Significant difference from CON1 ($p \leq 0.05$)
[c] Significant difference from CON1 ($p \leq 0.01$)
[d] Significant difference from CON2 ($p \leq 0.05$)

b. Body weight gains: Body weight gains for selected intervals are summarized in Table 4. For high-dose males, body weight gains were lower than control values for the entire study. Differences were statistically significant from both controls for Weeks 0 through 12 and for Week 0 to 18, 20 and 22; significant differences from the first control only were noted for Weeks 0 to 24. Body weight gains for high-dose females were significantly lower than both controls for Weeks 0 to 7, 11, 12, 14, 16, and 78; significant differences from the first control were noted for Weeks 0 to 13, 18, 22, 26 through 76, and 80 through 104. Weight gains were significantly lower than the second control for Weeks 0 to 3. Occasional significant differences from one, but not both, controls were noted in the mid-dose males and females.

Table 4: Mean Body Weight Gains (g) (Data summarized from Tables 6 and 8 of study)

Week	0 ppm (CON1)	300 ppm	750 ppm	1500 ppm	0 ppm (CON2)
Males					
0 - 1	47.6	47.7	47.0	42.0[M]	48.6
0 - 2	83.0	85.2	84.0	78.0[M]	85.8
0 - 4	136.2	137.6	137.6	125.6[M]	137.4
0 - 8	217.0	215.0	215.5	201.3[M]	215.5
0 - 12	261.9	259.2	261.1	243.1[M]	257.9
0 - 16	292.7	294.1	291.3	273.7	291.0
0 - 24	333.9	330.0	327.8	305.5[b]	321.5
0 - 32	361.3	359.9	361.5	335.8	359.2
0 - 40	395.0	395.8	398.0	373.9	393.7
0 - 56	442.1	437.9	441.8	413.9	440.6
0 - 72	469.4	460.9	465.1	435.7	461.7
0 - 88	494.3	472.7	468.6	426.7	455.2
0 - 104	432.5	414.6	434.7[a]	413.5	425.3
Females					
0 - 1	19.8	20.2	21.7	20.1	21.7
0 - 2	37.4	37.2	38.3	35.3	37.7
0 - 4	64.5	65.1	65.7	58.6[M]	64.1
0 - 8	95.4	95.6	96.4	88.6	95.3
0 - 12	111.5	112.1	112.6	103.0[M]	110.3
0 - 16	128.7	126.9	129.8	116.2[M]	126.0
0 - 24	149.2	145.4	151.5	133.5[M]	144.6
0 - 32	177.5	169.1	179.0	157.7[b]	168.3
0 - 40	205.7	197.9	206.4	178.9[b]	193.8
0 - 56	244.7	239.6	251.4	219.5	234.3
0 - 72	277.1	274.2	290.2	248.3[b]	262.1
0 - 88	320.8	310.7	341.7	277.3[b]	306.4
0 - 104	314.3	303.6	323.9[a]	251.6[b]	274.0

[a] Significant difference from CON1 ($p \leq 0.05$)
[b] Significant difference from CON1 ($p \leq 0.01$)
[c] Significant difference from CON2 ($p \leq 0.05$)
[d] Significant difference from CON2 ($p \leq 0.01$)

5. Food Consumption: Food consumption was measured at weekly intervals through Week 14 and every other week, thereafter. Statistically significant differences in food consumption were noted in both males and females throughout the study (Table 5, below). The occurrence was, however, sporadic in nature and therefore, did not to appear to be a treatment-related effect.

Table 5: Food Consumption (g/animals/day) (Data summarized from Tables 9 and 10 of study)

Week	0 ppm (CON1)	300 ppm	750 ppm	1500 ppm	0 ppm (CON2)
Males					
0 - 1	24.5	24.2[d]	24.0[d]	23.2[M]	25.5[b]
3 - 4	26.5	26.5	24.9	24.1[M]	25.2[b]
4 - 5	25.5	26.4[c]	25.0	23.4[M]	25.1
5 - 6	25.6	25.3	24.5[b]	23.3[M]	25.2
6 - 7	25.8	25.0[d]	24.9[c]	23.7[M]	25.8
7 - 8	26.0	25.1	25.4	24.2[M]	26.1
8 - 9	25.0	24.9	25.0	24.1[M]	25.3
9 - 10	25.4	25.0	25.0	24.2[M]	25.7
10 - 11	25.4	25.3	25.3	24.2[M]	25.5
11 - 12	25.8	25.0[M]	25.1	24.6[M]	26.0
12 - 13	24.9	24.6[c]	24.6[c]	24.3[d]	25.6
15 - 16	26.6	27.0[d]	26.2	25.2[b]	25.9
35 - 36	24.8	25.5	25.5	24.4[c]	25.4
41 - 42	26.2	26.3	26.5	25.1[M]	26.1
45 - 46	26.1	26.0	26.6	25.4[d]	26.8
55 - 56	26.2	25.7	25.6	24.73[b]	25.1
65 - 66	26.9	26.5	27.0	25.1[c]	25.8
69 - 70	26.5	25.6	26.1	24.8[M]	26.5
87 - 88	25.5	24.1	24.2	22.3[M]	24.9
97 - 98	25.1	23.7[d]	24.3[d]	24.1[d]	27.7[b]
Females					
0 - 1	17.5	17.4	17.1	15.9[M]	17.2
2 - 3	18.1	17.8	18.2	17.2[M]	18.3
3 - 4	18.3	18.7	18.4	17.2[M]	18.4
4 - 5	18.4	17.9	18.0	17.0[M]	18.1
5 - 6	18.0	17.9	17.8	17.1[M]	18.3
6 - 7	17.7	16.9[M]	17.8	17.1[M]	17.8
8 - 9	17.3	17.7	18.1[M]	18.2[M]	17.2
11 - 12	18.1	17.7	17.6	17.2[b]	17.2[b]
12 - 13	17.8	17.2[c]	17.6	17.0[b]	17.3[b]
15 - 16	18.2	17.3[c]	18.6[c]	17.2[c]	17.8
17 - 18	18.6	17.9[c]	18.6	17.7[M]	18.5
25 - 26	19.7	18.9[c]	19.2	17.7[M]	18.8[c]
27 - 28	19.5	19.3	19.8[c]	18.6[c]	18.8
35 - 36	19.2	19.8	19.7[c]	18.2[c]	18.6
41 - 42	21.2	20.1[c]	20.9	19.4[M]	20.7
47 - 48	21.4	20.9	21.2[c]	19.8[b]	20.6[b]
69 - 70	21.9	21.6[d]	21.4[c]	20.8[c]	19.3[c]
75 - 76	21.6	21.2[c]	21.7[d]	19.8[c]	19.4[c]

[a] Significant difference from CON1 ($p \leq 0.05$)
[b] Significant difference from CON1 ($p \leq 0.01$)
[c] Significant difference from CON2 ($p \leq 0.05$)
[d] Significant difference from CON2 ($p \leq 0.01$)

5. Achieved Compound Intake: The overall mean value for achieved compound intake is summarized in Table 6.

Table 6: Compound Intake (mg/kg/day) for Weeks 0 to 104 (Data taken from page 17 of study)

Sex	300 ppm	750 ppm	1500 ppm
Male	13	32	64
Female	16	41	83

6. Clinical Pathology: Hematology and clinical chemistry evaluations were performed on selected animals (15 animals/sex/group) from each study group during Weeks 26, 52, 78 and 104 of the study. Blood was collected by retroorbital bleeding after an overnight (16 - 24 hrs) fast. Urine samples were collected for a 24-hour interval during Weeks 25, 51, 77 and 103; food and water were available *ad libitum* during the collection. No hematology or clinical chemistry historical control values were included with the study.

a. Hematology: The hematological parameters listed below were evaluated during the study. No consistent effects attributable to treatment were noted during the study for either males or females.

Hematocrit	Reticulocyte count
Hemoglobin	Leukocyte differential count
Leukocyte count	Mean corpuscular hemoglobin
Platelet count	Mean corpuscular hemoglobin conc.
Erythrocyte count	Mean corpuscular volume

Although occasional, statistically significant findings were observed, the magnitude of the responses were not suggestive of biologically significant effects.

b. Clinical Chemistry: The clinical chemistry parameters listed below were evaluated during the study.

Electrolytes
- Calcium
- Chloride
- Phosphorous
- Potassium
- Sodium

Enzymes
- Alkaline phosphatase (ALP)
- Creatinine phosphokinase (CK)
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- γ-Glutamyl transpeptidase

Other
- Albumin
- Blood creatinine
- Blood urea nitrogen
- Total cholesterol
- Globulins
- Glucose
- Total Bilirubin
- Total Protein
- Direct bilirubin
- Indirect bilirubin
- A/G Ratio

Although occasional significant differences were noted, the magnitude of the responses were not great enough to be of biological significance.

c. Urinalysis: The following parameters were measured during the study:

Total volume	Glucose
Specific gravity	Ketone bodies
Protein	Occult blood
Appearance	Urobilirubin
Sediment	Total bilirubin
pH	Specific gravity
Color	

The only significant findings occurred at Week 77, where the high-dose females showed a decreases in total urine volume accompanied by an increase in specific gravity.

7. Ophthalmological examinations: Examinations were performed during the prestudy acclimation period and again before terminal sacrifice. No treatment-related eye lesions were observed. During the prestudy evaluation, a high percentage of the animals exhibited corneal crystals. The corneal crystals were also present at terminal sacrifice, with high incidences in all of the dose groups. The study states that the presence of corneal crystals is common for the strain and age of the animals used in the study.

8. Sacrifice and Pathology: The tissues listed below were fixed in 10% neutral buffered formalin. Tissues from the two control groups and the high-dose group were examined histologically, while the lungs, livers, kidneys and gross lesions were examined for all dose groups. Selected organs (CAPITAL LETTERS) were also weighed. Terminal body weights were also recorded.

Digestive system
- Pancreas
- Salivary gland
- Esophagus
- Stomach
- Duodenum
- Jejunum
- Ileum
- Cecum
- Colon
- Rectum
- LIVER
- Gall Bladder

Respiratory
- Lungs
- Trachea

Cardiovas./Hematol
- Aorta
- HEART
- Bone marrow
- Lymph nodes
- SPLEEN
- Thymus

Urogenital
- KIDNEYS
- Urinary bladder
- TESTES
- Ovaries
- Prostate
- Uterus
- Epididymis
- Vagina
- Seminal vesicles

Neurologic
- BRAIN with stem
- Periph. nerve
- Spinal cord
- Pituitary
- Eyes

Glandular
- Adrenals
- Thyroids
- Mammary gland

Other
- Gross lesions
- Skin
- Bone
- Skeletal muscle

a. Organ Weights: At terminal sacrifice, body weights and selected organs weighs were determined; significant findings are summarized in Table 7. Significant differences (from control 1) were limited to high-dose females and consisted of decreased terminal body weight and increased brain weight, relative to terminal body weight. The higher relative brain weight appears to be a reflection of decreased terminal body weight rather than a treatment-related effect.

Table 7: Terminal Body Weights and Relative Organ Weights (Data summarized from Appendix J, Tables 1, 4 and 5 of study)

	0 ppm (CON1)	300 ppm	750 ppm	1500 ppm	0 ppm (CON2)
Terminal Body Weights, g					
Male	674	656 (-2.8,-1.5)	688 (2.1,3.4)	656 (-2.9,-1.6)	664
Female	481	459 (-4.6,5.0)	479 (-0.4,9.6)	407* (-15.4,-6.9)	437
Brain Weight Relative to Terminal Body Weight, %					
Female	0.438	0.451	0.436	0.514*	0.484

[1] Values in parentheses represent the percent of control values (% of CON1,% of CON2)

* Significant difference from CON1 ($p \leq 0.01$)

b. Gross Pathology: Gross necropsy examination of tissues from animals found dead, sacrificed *in extremis*, or at termination of the study did not reveal any treatment-related findings.

c. Microscopic Pathology: Significant nonneoplastic lesions are summarized in Table 8. At terminal sacrifice, the mesenteric lymph node of high-dose males and females showed increased incidence of blood in the sinuses, hemosiderosis and histiocytosis. Additionally, high-dose females had an increased incidence of bile duct hyperplasia and mononuclear infiltration of the liver.

Although neoplastic lesions were noted, the incidences were comparable among all study groups and not suggestive of any treatment-related effects. A summary of neoplastic lesions is enclosed (Appendix 1).

Table 8: Histopathological Findings (Data taken from Appendix 3, Tables 11 to 24 study)

Observation	0 ppm (CON1)	300 ppm	750 ppm	1500 ppm	0 ppm (CON2)
Mesenteric Lymph Nodes - Males					
Total number examined	57[1] (36, 21)	4 (1, 3)	5 (3, 2)	57 (33, 24)	54 (39, 15)
Blood in sinuses	1 (1, 0)	2 (1, 1)	1 (0, 1)	11 (8[**], 3)	3 (1, 2)
Hemosiderosis	2 (2, 0)	2 (0, 2)	2 (2, 0)	19 (12[**], 7)	2 (1, 1)
Histiocytosis	6 (5, 1)	0 (0, 0)	0 (0, 0)	19 (10[*], 9)	5 (2, 3)
Mesenteric Lymph Nodes - Females					
Total number examined	56 (28, 28)	4 (4, 0)	4 (3, 1)	57 (35, 22)	54 (27, 27)
Blood in sinuses	2 (1, 1)	2 (2, -)	1 (1, 0)	12 (7[*], 5)	6 (4, 2)
Hemosiderosis	6 (2, 4)	1 (1, -)	2 (1, 1)	25 (16[$], 9)	8 (7, 1)
Histiocytosis	12 (4, 8)	2 (2, -)	1 (1, 0)	31 (18[$], 13)	17 (10, 7)
Liver - Females					
Total number examined	60 (30, 30)	60 (36, 24)	59 (38, 21)	60 (38, 22)	60 (30, 30)
Bile duct hyperplasia	27 (16, 11)	29 (22, 7)	27 (21, 6)	40 (30[*], 10)	27 (19, 8)
Mononuclear infiltration	11 (6, 5)	10 (6, 4)	14 (14, 0)	17 (16[*], 1)	10 (5, 5)

[1] Values represent lesion incidence for all animals on study. Numbers in parentheses (X, Y) represent incidence at terminal sacrifice (X) and animals found dead or moribund sacrifices (Y).

[*] Significant difference from CON1 ($p \leq 0.05$)
[**] Significant difference from CON1 ($p \leq 0.01$)
[$] Significant difference from CON2 ($p \leq 0.05$)
[$$] Significant difference from CON2 ($p \leq 0.01$)

E. DISCUSSION: In this combined chronic toxicity/oncogenicity study, male and female rats were fed diets containing 0, 300, 750 or 1500 ppm test compound (mg/kg/day equivalents: 0, 13, 32, or 64 for males and 0, 16, 41, or 83 for females). No treatment-related effects were noted in either the incidence of clinical signs or deaths. The percent mortalities (excluding accidental deaths) and mean survival times were comparable among all of the study groups.

Although significant decreases were noted in the mean body weights of high-dose males and females, the differences were slight with percent changes generally less than 10% (0.4 to 6.5%, males; 0.6 to 7.3%, females). The significant decreases in food consumption by high-dose animals appears to be a reflection of decreased body weight rather than a treatment-related effect. Hematology, clinical chemistry and urinalysis findings of treated animals were comparable to those of the control.

The incidence of gross pathological findings did not reveal any treatment-related effects. Histopathological evaluation revealed significant increases in the incidence sinusoidal blood, hemosiderosis and histiocytosis in the mesenteric lymph nodes of high-dose males and females. High-dose females also had an increased incidence of bile duct hyperplasia and mononuclear infiltration of the liver.

In a parallel study (MRID No.: 418023-01), male and female CD-1 mice were fed diets containing BARDAC 2280 at concentrations of 0, 100, 500, or 1000 ppm (mg/kg/day equivalents: 0, 15.0, 76.3, or 155.5 for males and 0, 18.6, 93.1, or 193.1 for females). No treatment-related effects were noted in the incidence of clinical signs, deaths, gross and histopathological observations. Hematological values were comparable among all study groups. Effects attributable to treatment included decreased mean body weights and body weight gains of high-dose males and females.

F. CONCLUSIONS

Adequacy of the High Dose Tested to Assess Chronic Toxicity and Carcinogenicity: Doses for this study were selected based on a 90-day study (MRID No. 409663-02) which identified a LOEL of 3000 ppm (175 mg/kg/day, males; 226 mg/kg/day, females). The LOEL (highest dose tested, HDT) was based on mortality (80%), decreases in mean body weighs, body weight gain and food consumption, alterations in hematology and clinical chemistry parameters, gross pathology (emaciation, hemorrhagic stomachs, decreased spleen size and dilated and distended cecum) and non-neoplastic lesions (glycogen depletion in the liver, splenic contraction, sinus erythrocytosis and lymphoid hyperplasia on the mesenteric lymph nodes).

In the present study, the HDT (1500 ppm = 64 mg/kg/day, males; 83 mg/kg/day, females) did not alter survival or result in adverse clinical signs. Animals showed only moderate weight loss. Although males and females showed statistically significant

decreases in mean body weights, the differences were slight (approximately 6%) and did not meet the weight gain depression criteria of 10%. However, this dose induced significantly higher incidences of non-neoplastic lesions in the mesenteric lymph nodes (sinusoidal blood, hemosiderosis and histiocytosis) in both males and females, compared to their respective controls.

These lesions are spontaneous in nature and commonly seen in aging or aged rats and are not considered life-threatening. However, exacerbation of these spontaneous lesions was seen in both sexes of rats found dead, sacrificed moribund and sacrificed at termination. The incidence of these lesions in control males and females were within the normal background range for this age/strain of rats. Therefore, the increased incidence of these lesions is attributed to treatment. Higher dosing might have further increased/exacerbated the incidence and/or severity of these lesions resulting in life-threatening toxicity (i.e. early mortality).

Although the toxic effects predicted from the results of the 90-day study did not materialize and no carcinogenic response was seen, the HDT selected probably could not have been much higher. A dose of 3000 ppm would have resulted in "excessive toxicity" as indicated by the results of the 90-day study. A dose of 2000 ppm may have caused "sufficient toxicity" resulting in additional decrements in body weight gain and exacerbation of the spontaneous non-neoplastic lesions. The HDT (1500 ppm) induced "minimal toxicity" without substantially altering the normal life-span of the animals, indicating that the Maximum Tolerated Dose was "barely" missed. Therefore, it is concluded that the HDT was adequate to assess the chronic toxicity and carcinogenic potential of BARDAC 2280.

Under the conditions of this study, for chronic toxicity a NOEL of 750 ppm (32 mg/kg/day, males; 41 mg/kg/day, females) and a LOEL of 1500 ppm (64 mg/kg/day, males; 83 mg/kg/day, females) is was established. The LOEL was based on moderate decreases in mean body weights and body weight gain and increased incidences of non-neoplastic lesions in the mesenteric lymph nodes (sinusoidal blood, hemosiderosis and histiocytosis). At the dose levels tested, BARDAC 2280 was not carcinogenic in male or female mice.

CORE CLASSIFICATION: Minimum This study satisfies guideline requirements (§83-5(a)) for a combined chronic/oncogenicity study in rats and is acceptable for regulatory purposes.

APPENDIX 1: SUMMARY OF NEOPLASTIC LESIONS (Taken from Appendix 3, Tables 14, 15, 17, 18, 19, and 20 of study)

Page_____ is not included in this copy.

Pages 123 through 145 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

_____ The document is not responsive to the request.

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

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FINAL

DATA EVALUATION REPORT

BARDAC 22

Study Type: Developmental Toxicity

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer: Pia Lindström, DPH — Date 11/17/93

Independent Reviewer: Sanju Diwan, Ph.D. — Date 11/17/93

QA/QC Manager: Sharon Segal, Ph.D. — Date 11/17/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 289
Project Officer: Caroline Gordon

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Guideline Series 83-3: Developmental Toxicit

EPA Reviewer: Robert Fricke, Ph.D. Signature: Robert F. Fricke
Review Section IV, Toxicology Branch II/HED Date: 18 Nov 93

EPA Section Head: Jess Rowland, M.S. Signature: Jess Rowland
Review Section IV, Toxicology Branch II/HED Date: 11/19/93

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity (Rat); Guideline Series 83-3 (a)

EPA IDENTIFICATION NUMBERS

DP BARCODES: D188679, D189620 SUBMISSION No.: S436173

PC CODE: 069149 CASWELL No.: 331A

MRID No.: 416867-01

TEST MATERIAL: Didecyldimethylammoniumchloride

SYNONYM: Bardac 22; DDAC

SPONSOR: Lonza Inc., Fair lawn, NJ

STUDY NUMBER: 53-534

TESTING FACILITY: Bushy Run Research Center, Export, PA

TITLE OF REPORT: Developmental Toxicity Evaluation of Didecyldimethyl-ammoniumchloride Administered by Gavage to CD® (Sprague-Dawley) Rats

AUTHOR: T.L. Neeper-Bradley

REPORT ISSUED: May 17, 1991

CONCLUSIONS: In a developmental toxicity study Crl:CD® rats were administered BARDAC 22 via gavage at doses of 0, 1, 10, or 20 mg/kg/day on gestational days (GDs) 6-15, inclusive.

Maternal NOEL = 1 mg/kg/day
Maternal LOEL = 10 mg/kg/day based on clinical signs including audible respiration

In addition, at 20 mg/kg/day, maternal toxicity was evident as clinical signs (audible respiration and gasping) and decreased weight gain and food consumption during the dosing period.

Developmental NOEL = 20 mg/kg/day
Developmental LOEL = Not determined

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CORE CLASSIFICATION: Minimum. This study meets the guideline requirements (83-3) for a developmental toxicity study in rats, and is acceptable for regulatory purposes.

A. MATERIALS

Test Compound

Purity:	80.8% Active ingredient
Description:	Viscous, honey-colored liquid
Lot number:	B-1889
Receipt date:	November 24, 1987
Contaminants:	None reported
Storage:	Not reported

Vehicle: Deionized Millipore® water

Test Animals

Species:	Rat
Strain:	Sprague-Dawley Crl:CD®BR
Source:	Charles River Laboratories, Portage, MI
Age:	Approximately 70 days on GD 0
Weight:	200-251 g on GD 0
Males used:	Same strain from the same source as the females

B. STUDY DESIGN

This study was designed to assess the potential of BARDAC 22 to cause developmental toxicity in rats when administered daily via gavage on GDs 6-15, inclusive.

Mating

Following approximately 2 weeks of acclimation, females were mated to males (in a ratio of 1:1) of the same strain used specifically for breeding. The day a copulatory plug was observed was designated GD 0.

Animal Husbandry

Food (Purina® Certified Ground Rodent Chow) and tap water were available ad libitum throughout the study. A 12/12-hour light/dark cycle was maintained. Temperature was maintained at 66-77°F and humidity was maintained at 40-70%.

Group Arrangement

Animals were assigned to the following dose groups using a computer-generated weight-stratified randomization procedure:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	25
Low-dose	1	25
Mid-dose	10	25
High-dose	20	25

Dose Administered

Doses were administered daily via gavage from GD 6 through 15 in a volu of 5 mL/kg. Individual doses were calculated based on the most recently recorded body weight data. Dosing solutions were prepared twice during the study and adjusted for active ingredient. They were analyzed for concentration prior to use. Analyses for stability and homogeneity were conducted prior to study initiation.

Dose Rationale

Doses were selected based upon results of a range-finding study (BNRC Report Number 83-533). The results of this study were not presented.

Observations

Animals were observed twice daily for mortality, moribundity, and clinical signs. Body weight data were recorded on GDs 0, 6, 9, 12, 15, 18, and 21. Food consumption data were recorded for three-day intervals during GDs 0-21. On GD 21 animals were sacrificed by carbon dioxide asphyxiation and litters were delivered by cesarean section. Examinatio of the dams at sacrifice included the following:

- Gross pathology of thoracic, abdominal, and pelvic cavities
- Examination of the lumen and lining of the esophagus, stomach and trachea for signs of irritation caused by the test material
- Gravid uterine weights
- Liver weights
- Number of corpora lutea
- Number of implantation sites
- Numbers of resorptions (early and late) and live and dead fetuses

Uteri from apparently nonpregnant animals were stained with 10% ammonium sulfide to detect early embryonic loss.

Examination of live fetuses included the following:

- Individual fetal weight and sex
- External anomalies including cleft palate
- Visceral anomalies for approximately one-half of the fetuses
- Craniofacial structures for the animals selected for visceral examination
- Skeletal anomalies for approximately one-half of the fetuses

Statistical Analysis

The following methods were used:

- Quantitative continuous data--Levene's test, ANOVA, and t-tests
- Nonparametric data--Kruskal-Wallis test, Mann-Whitney U test
- Incidence data--Fisher's Exact test

Compliance

- A signed Statement of No Data Confidentiality Claims, dated April 26, 1991, was provided.
- A signed Statement of Compliance with EPA GLPs, dated April 24 and 27, 1991, was provided.
- A signed Quality Assurance Statement, dated May 17, 1991, was provided.
- The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

C. RESULTS

Test Material Analysis

Concentration of dosing solutions ranged from 97 to 104% of target. Analysis for homogeneity ranged from 87 to 108% of target. Analysis for stability ranged from 92 to 101% of target after 12 or 15 days at room temperature.

Maternal Toxicity

Mortality: No mortality was observed at any dose level.

Abortion: No abortions were reported at any dose level.

Clinical observations: Compound-related clinical signs were observed at 20 and 10 mg/kg/day. A summary of selected clinical signs is presented in Table 1. At 20 mg/kg/day, these signs included audible respiration and gasping; at 10 mg/kg/day, they were limited to audible respiration.

Body weight: No significant compound-related effects on body weight/weight gain were observed at any dose level. A summary of body weight gain for selected intervals is presented in Table 2. There was, however, a biologically significant decrease (67%; nonsignificant) in weight gain during the dosing period at 20 mg/kg/day. This was mainly due to decreases on GDs 9-12 and 12-15 (54 and 74%, respectively; data not shown). Corrected weight gain also decreased nonsignificantly (88%) at this dose level.

Food consumption: No significant compound-related effects on food consumption (g/animal/day) were observed at any dose level (data not shown). However, decreases were noted at 20 mg/kg/day on GDs 9-12 (91%), 12-15 (88%), and 6-15 (93%)

Gross pathology observations: No compound-related gross pathology was observed.

Organ Weights: No compound-related effects on maternal liver weights were observed at any dose level.

Cesarean section observations: No compound-related effects were observed at any dose level in any parameter. A summary of cesarean section data is presented in Table 3.

Developmental Toxicity

No compound-related developmental toxicity was observed at any dose level. A summary of gross, visceral, and skeletal malformations is presented in Table 4.

External examinations: External malformations consisted of one fetus in the control group with imperforate anus and thread-like tail and a second fetus from the same litter with a shortened foot; one edematous fetus at 10 mg/kg/day; and two fetuses from different litters at 20 mg/kg/day, one with micrognathia and missing eye bulge and one with an umbilical hernia. External variations, occurring with similar incidences in all dose groups, were limited to ecchymosis.

Visceral examinations: Visceral malformations consisted of three fetuses from different litters in the control group with hydronephrosis and/or hydroureters. At 1 mg/kg/day, six fetuses from four litters had hydroureters. At 10 mg/kg/day, 14 fetuses from seven litters had hydronephrosis and/or hydroureters. At 20 mg/kg/day, seven fetuses from six litters had hydroureters and/or umbilical hernia. Visceral variations, occurring with similar incidences in all dose groups, included fetal atelectasis, dilated renal pelvis, and dilated ureter.

Skeletal examinations: Skeletal malformations consisted of three fetuses from two litters in the control group, two (two litters) with multiple malformations and one with missing cervical arches; at 10 mg/kg/day, two

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fetuses from different litters with missing ribs; and at 20/mg/kg/day, two fetuses from different litters with multiple malformations. Skeletal variations, occurring with similar incidences in all dose groups, included poor or no ossification of various bones and bilobed cervical and thoracic centra.

D. DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) to be included with the evaluation of the study. All criteria were satisfied.

Test Material Analyses

Analyses for test material concentration revealed values within ±6% of nominals. Stability analysis demonstrated that the test material was stable in the vehicle for 15 days at room temperature. Homogeneity of the test material in the vehicle was demonstrated.

Maternal Toxicity

Compound-related and dose-dependent maternal toxicity was observed at 10 and 20 mg/kg/day. It was manifested as significantly increased clinical signs (audible respiration and/or gasping) at both dose levels. At 20 mg/kg/day, biologically but not statistically significant decreases (frequently >10%) in weight gain and food consumption were noted during dosing. Based on these results, the NOEL and LOEL for maternal toxicity were 1 and 10 mg/kg/day, respectively.

Developmental Toxicity

No developmental toxicity (including deaths/resorptions, altered growth, and developmental anomalies [by category or total malformations]) was observed at any dose level. Consequently, the NOEL for developmental toxicity was 20 mg/kg/day. The LOEL was not determined.

Maternal NOEL = 1 mg/kg/day
Maternal LOEL = 10 mg/kg/day (based on increased clinical signs)

Developmental Toxicity NOEL = 20 mg/kg/day
Developmental Toxicity LOEL = Not determined

E. CORE CLASSIFICATION: Minimum. This study meets the guideline requirements (83-3) for a developmental toxicity study in rats, and is acceptable for regulatory purposes.

F. RISK ASSESSMENT: Not applicable

Table 1. Summary of Selected Clinical Signs During Dosing[a]

Observation	Dose Level (mg/kg/day)			
	0	1.0	10.0	20.0
Number of animals	25	25	25	25
Unkempt body	0	0	0	2
Urine stain	0	0	0	1
Red urogenital discharge	1	0	0	0
Audible respiration	0	0	6*	14**
Gasping	0	0	0	4
Perinasal encrustation	0	0	0	3
Loose feces	0	0	0	2
Perioral encrustation	0	0	0	1

[a]Data were extracted from Study No. 53-53(, Table 2.

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

TABLE 2. Mean Body Weight Gain (g ± S.D.)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GDs 0-6)	Dosing Period (GDs 6-15)	Post-Dosing Period (GDs 15-21)	Gestation Period (GDs 0-21)	Corrected Body Weight Gain (GDs 0-21)
0	28 ± 5.3	36 ± 5.8	86 ± 11.1	150 ± 19.1	52 ± 13.5
1.0	30 ± 6.5	40 ± 5.5	90 ± 9.1	159 ± 16.7	57 ± 13.4
10.0	29 ± 5.9	34 ± 8.3	87 ± 12.7	150 ± 22.1	53 ± 16.7
20.0	29 ± 6.0	25 ± 23.7	87 ± 13.5	141 ± 32.8	[illegible] ± 25.5

[a]Data were extracted from Study No. 53-534, Tables 3 and 4.

TABLE 3. Cesarean Section Observations[a]

Parameter	Dose level (mg/kg/day) 0	1.0	10.0	20.0
No. animals mated	25	25	25	25
No. animals pregnant	25	24	24	23
Pregnancy rate (%)[c]	100	96	96	92
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	0	1	1	2
No. aborted	0	0	0	0
Gravid uterine weight (g)	98.2	102.6	97.0	95.1
Dams with live litters	25	24	24	23
Total corpora lutea	354	353	346	332
Corpora lutea/dam	14.2 ± 1.9[b]	14.7 ± 1.9	14.4 ± 1.9	14.4 ± 1.7
Total implantations	342	344	339	322
Implantations/dam	13.7 ± [illegible]	14.3 ± 1.7	14.1 ± 1.7	14.0 ± 1.5
Total live fetuses	[illegible]	337	319	311
Live fetuses/dam	[illegible]	14.0 ± 1.7	13.3 ± 2.1	13.5 ± 1.5
Total resorptions	5	7	19	11
Early resorptions	5	6	18	10
Late resorptions	0	1	1	1
Resorptions/dam[c]	0.2 ± 0.4	0.3 ± 0.5	0.8 ± 1.3	0.5 ± 0.6
Total dead fetuses	0	0	1	0
Dead fetuses/dam	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
Fetal weight/litter (g)	5.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.2	5.0 ± 0.6
Preimplantation loss (%)	4	3	3	5
Postimplantation loss (%)[c]	1	2	6	3
Sex ratio (% male)	52	52	48	47

[a]Data were extracted from Study No. 53-534, Tables 1 and 7 and Appendix 3, Table 3.

[b]Mean ± S.D.

[c]Calculated by the reviewers; not statistically analyzed

TABLE 4. Incidences of Fetal Malformations[a]

Findings[b]	Dose level (mg/kg/day) 0	1.0	10.0	20.0
External Malformations				
No. fetuses (litters) examined	337 (25)	337 (24)	319 (24)	311 (23)
Thread-like tail	1	0	0	0
Imperforate anus	1	0	0	0
Umbilical hernia	0	0	0	1
Micrognathia	0	0	0	1
Eye bulge missing	0	0	0	1
Fetus edematous	0	0	1	0
Foot shortened	1	0	0	0
Total No. fetuses (litters) with any external malformation	2 (1)	0	1	2 (2)
Visceral Malformations				
No. fetuses (litters) examined	176 (25)	175 (24)	164 (24)	162 (23)
Hydronephrosis-bilateral	1	0	1	0
Hydronephrosis-unilateral	0	0	1	0
Hydroureter-bilateral	2 (2)	3 (2)	12 (5)	4 (3)
Hydroureter-unilateral	1	3 (3)	2 (2)	2 (2)
Umbilical hernia	0	0	0	1
Total No. fetuses (litters) with any visceral malformation	3 (3)	6 (4)	14 (7)	7 (6)
Skeletal Malformations				
No. fetuses (litters) examined	161 (25)	162 (24)	155 (24)	149 (23)
Cervical centra missing	1	0	0	0
Cervical arches missing	2 (2)	0	0	0
All thoracic and lumber centra and arches missing	1	0	0	0
Lumber centrum and arch #6 missing	0	0	0	1
All sacral and caudal centra and arches missing	1	0	0	0
Missing rib #13	0	0	2 (2)	0
All ribs missing	1	0	0	0
Nasal fused and misshapen	0	0	0	1
Premaxillary fused and misshapen	0	0	0	1
Mandible fused	0	0	0	1
Some proximal and/or distal phalanges missing	1	0	0	0
Total No. fetuses (litters) with any skeletal malformation	3 (2)	0	2 (2)	2 (2)
Total No. fetuses (litters) with any malformation	6 (5)	6 (4)	16 (9)	9 (6)

[a]Data were extracted from Study No. 3147.54, Table 9 and Appendix 4.

[b]More than one type of malformation may be found in one fetus.

ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group).
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with an * are supplemental, may not be required for every study.

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010689

FINAL

DATA EVALUATION REPORT

BARDAC 22

Study Type: Developmental Toxicity

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer: Sanju Diwan, Ph.D. Date 11/16/93

Independent Reviewer: Pia Lindstrom, DPH Date 11/16/93

QA/QC Manager: Sharon Segal, Ph.D. Date 11/16/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 288
Project Officer: Caroline Gordon

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Guideline Series 83-3: Developmental Toxicity

EPA Reviewer: Robert Fricke, Ph.D.
Review Section IV, Toxicology Branch II/HED
Signature: Robert J. Fricke
Date: 12 Feb 93

EPA Section Head: Jess Rowland, M.S.
Review Section IV, Toxicology Branch II/HED
Signature: Jess Rowland
Date: 2/22/93

DATA EVALUATION REPORT

STUDY TYPE: Developmental toxicity (rabbits); Guideline Series 83-3

EPA IDENTIFICATION NUMBERS

DP BARCODES: D188679, D189620
SUBMISSION NO.: S436173
P.C. CODE: 069149
CASWELL NO.: 33EA
MRID No.: 410187-01

TEST MATERIAL: BARDAC 22

SYNONYMS: Didecyldimethylammoniumchloride

SPONSOR: Lonza Inc., Fair Lawn, NJ

STUDY NUMBER: 51-590

TESTING FACILITY: Bushy Run Research Center (BRRC), Export, PA

TITLE OF REPORT: Developmental Toxicity Study of Didecyldimethylammonium-chloride Administered by Gavage to New Zealand White Rabbits

AUTHOR: R.W. Tyl

REPORT ISSUED: January 27, 1989

CONCLUSIONS: In a developmental toxicity study, New Zealand White rabbits were administered BARDAC 22 daily via gavage at dose levels of 0, 1, 3, or 10 mg/kg/day on gestational days (GDs) 6-18, inclusively.

Maternal NOEL = 1 mg/kg/day
Maternal LOEL = 3 mg/kg/day based on clinical signs (hypoactivity, labored and/or audible respiration) and decreased body weight gain during the dosing period

In addition, at 10 mg/kg/day, there was an increased incidence of mortality, clinical signs (hypoactivity, labored and/or audible respiration) and decreased body weight gain during the dosing period.

Developmental NOEL = 3mg/kg/day
Developmental LOEL = 10 mg/kg/day based on decreased fetal body weight and increased number of dead fetuses

CORE CLASSIFICATION: Minimum. This study satisfies guideline requirements (83-3b) for a developmental toxicity study in rabbits and is acceptable for regulatory purposes.

A. MATERIALS

Test Compound

Purity:	81%
Description:	Honey-colored, viscous liquid
Batch number:	B-1889
Lot number:	1889
Receipt date:	August 13, 1987
Contaminants:	Not reported
Storage:	At room temperature

Vehicle: Millipore® filtered water (deionized)

Test Animals

Species:	Rabbit
Strain:	New Zealand White
Source:	Hazleton-Dutchland Laboratories, Inc., Denver, PA
Age:	Approximately 6 months on GD 0
Weight:	3.2-4.1 kg on GD 0
Males used:	Same strain from the same supplier

B. STUDY DESIGN

This study was designed to assess the potential of BARDAC 22 to cause developmental toxicity in New Zealand White rabbits when administered daily via gavage on GDs 6-18, inclusively.

Mating: Following approximately 2 weeks of acclimation, females were mated to males (1:1) of proven fertility. The day of copulation was designated GD 0.

Animal husbandry: Feed (Agway PROLAB Certified Chow®) and municipal tap water were available ad libitum throughout the study and were analyzed for contaminants. A 12-hour light/dark cycle was maintained. Temperature and humidity ranges were 66-72°F and 40-60%, respectively; frequency of air changes was not reported.

Group arrangement: Mated females were assigned to study groups, based on body weight on GD 0 using a weight-stratified randomization procedure, as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	16
Low-dose	1	16
Mid-dose	3	16
High-dose	10	16

Dose preparation: Doses were administered daily via gavage from GD 6 through 18 in a volume of 2.0 mL/kg of body weight. Individual doses were calculated based on the most recently recorded body weight data. The doses were adjusted for percent active ingredient. Dosing solutions were prepared once, analyzed for concentrations and stored at room temperature. The homogeneity of the lowest and highest doses was determined prior to initiation of dosing; stability was determined after 7, 14, and 21 days concurrently with the dosing period.

Dose rationale: Dose levels were selected based on the results of a dose range-finding study (BRRC Report 51-525); however, the results were not provided.

Observations: Animals were observed twice daily for mortality and moribundity and at least once daily for clinical signs. Body weight data were recorded on GDs 0, 6, 13, 19, 24, and 29. Food consumption data were not recorded. On GD 29, does were euthanized by intravenous injection of T-61 euthanasia solution and litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

- Gross pathology examination of thoracic, abdominal, and peritoneal cavities and reproductive organs
- Liver and gravid uterine weights
- Number of corpora lutea and implantation sites
- Number of resorptions (early and late)
- Number of live and dead fetuses

The uteri of apparently nonpregnant does were stained with a 10% aqueous solution of ammonium sulfide to detect early embryo loss.

All fetuses were examined in the following manner:

- Individual fetal weight and sex

- External anomalies
- Craniofacial structures of one-half of the live fetuses (heads fixed in Bouin's solution)
- Visceral anomalies
- Skeletal anomalies using the Alizarin red S staining method

Statistical analysis: The following methods were used.

- Parametric data-- Levene's test for equal variances, ANOVA, and t-tests
- Nonparametric data-- Kruskal-Wallis test and Mann-Whitney U-test
- Incidence data-- Fisher's Exact test

Regulatory Compliances

- A signed Statement of No Data Confidentiality Claim, dated November 18, 1988, was provided.
- A signed Statement of Compliance with FIFRA GLPs, dated October 26 and November 18, 1988, was provided.
- A signed Quality Assurance Statement, dated January 27, 1989, was provided.
- A signed FIFRA Flagging Statement, dated February 9, 1989, was provided.

C. RESULTS

Test Material Analysis

Mean concentration analyses of the dosing solutions ranged from 94% to 101% of target for all three concentrations. Stability of the test compound in the low- and high-dose solutions over 21 days ranged from 94% to 104% of nominal values. Homogeneity of the dosing solutions ranged from 90% to 110% of target.

Maternal Toxicity

Mortality/morbundity: Compound-related mortality was observed at 10 mg/kg/day. Four of 16 does (25%) from this dose group died prior to GD 12 (GDs: 9, 9, 10 and 11). Necropsy (data not shown) revealed the following in some or all animals that died: sloughing of the esophageal lining; sloughing, hemorrhage and/or distension of the glandular and nonglandular portion of the stomach; color change, firm texture, and/or consolidation of the lungs; gas filled intestines, reticular pattern in the liver and distended urinary bladder; and trachea with mucoid fluid or material.

Abortions: No abortions were noted. One and two does at 10 and 1 mg/kg/day, respectively, delivered early and were consequently excluded from the study.

Clinical observations: Compound-related maternal clinical signs were observed in does at 3 and 10 mg/kg/day. A summary of clinical signs observed during the dosing period is presented in Table 1. Labored/audible respiration, hyperactivity, gasping, and loose feces we not observed in controls and therefore, were considered to be compound related.

Body weight: Compound-related effects on weight gain were observed a 3 and 10 mg/kg/day. A summary of maternal body weight gain for selec intervals is presented in Table 2.

Body weight did not differ significantly amoung groups at any time, although at 10 mg/kg/day it decreased below control by 10% and 9% on days 19 and 24, respectively (data not shown). Body weight gain decreased significantly during the dosing period at 3 and 10 mg/kg/day and increased nonsignificantly during the postdosing period at 10 mg/kg/day (Table 2). Overall weight gain during the entire gestat was only 68% and 61% of control at 3 and 10 mg/kg/day, respectively (Table 2). A closer evaluation of weight gain data during the dosing period (GDs 6-19), revealed significant decreases at 10 mg/kg/day on GDs 6-13 and 13-19 and at 3 mg/kg/day on GDs 13-19 (data not shown). closer evaluation of weight gain data during the postdosing period (GDs 19-29), revealed a significant increase at 10 mg/kg/day on GDs 24-29 (data not shown). Corrected body weight gain decreased significantly (>2 fold) at 3 and 10 mg/kg/day (Table 2).

Necropsy observations: No compound-related necropsy findings were observed in any dose group at scheduled necropsy (data not shown).

Cesarean section observations: Compound-related effects were observed at 10 mg/kg/day. A summary of cesarean section data is presented in Table 3.

At 10 mg/kg/day, a significant increase in the number of dead fetuses per litter was observed compared to control. The number of dead fetus did not increase in a dose-related manner. However, in the absence of historical control data that would demonstrate the opposite, the reviewers consider this finding to be compound related. The mean feta body weights (34.88-35.77 g) were lower (13%; nonsignificant) than controls and were outside the range for the historical controls (≥40 g Therefore, this decrease was considered to be compound related.

Developmental Toxicity

No compound-related anomalies were observed at any dose level. A summary of selected external, visceral, and skeletal malformations is presented in Table 4.

External examinations: One fetus at 10 mg/kg/day exhibited multiple malformations including clubbed limbs, scoliosis, and short tail (Table 4). Another two fetuses from one litter had rigid joints. Three fetuses from different litters at 3 mg/kg/day exhibited a single malformation each (gastroschisis, umbilical hernia, and dome-shaped head). One additional fetus at 1 mg/kg/day also exhibited gastroschisis. Variations occurring in all dose groups, included ecchymosis of head and/or trunk and extremities and dome-shaped head (data not shown).

Visceral examinations: One, four (one litter), five (4 litters), and one fetus(es) from control, 1-, 3-, and 10-mg/kg/day dose groups, respectively, had a dilated lateral ventricle with depressed tissue (Table 4); a kidney was missing from the same fetus (as above) from the 10-mg/kg/day group. Six fetuses (5 litters) from the control group and three fetuses (3 litters) each from the 3- and 10-mg/kg/day dose groups had missing azygous lung lobes. Among two control fetuses from separate litters, one had a short ureter and another had a missing urinary bladder. At 1 mg/kg/day, one fetus had a missing ovary while another had a missing uterine horn. The gall bladder was missing from two fetuses (one litter) and one fetus at 1 and 3 mg/kg/day, respectively. Other incidental findings included umbilical hernia in one fetus at 3 mg/kg/day and gastroschisis in one fetus each at 1 and 3 mg/kg/day. The incidence of none of these malformations was significantly higher than control. Variations, occurring in all dose groups, consisted of heart, ventricles, liver, and stomach in addition to fetal atelectasis (data not shown).

Skeletal examinations: One fetus at 10 mg/kg/day exhibited multiple skeletal malformations which involved thoracic and lumber centra or arches, sacral vertebrae, and ribs (Table 4). In addition, lateral scoliosis was seen in 2 fetuses (2 litters) in the control and 2 fetuses (1 litter) in the 10-mg/kg/day dose group. Extra lumbar arch #8 was seen in one fetus each from a control and 1-mg/kg/day dose groups; in addition one fetus from the later group also had an extra lumbar centrum #8. Duplicated sternebrae were seen in one fetus each from control and 3-mg/kg/day dose groups. Variations, occurring in all dose groups, were noted in the cervical and thoracic centra, lumbar arches, caudal vertebra, ribs #13, frontal, parietal, hyoid and pubis bones, sternebrae, scapulae, phalanges and metacarpals and metatarsals (data not shown).

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment 1) to be included with the evaluation of the study. Criterion #9 (food consumption) was not satisfied.

Test Material Analyses

The purity of the test compound was confirmed. Concentration, homogeneity and stability analyses of the test compound in the vehicle revealed values within the acceptable range of ±10% of target.

Maternal Toxicity

Compound-related maternal toxicity was observed at 3 and 10 mg/kg/day. It was manifested as increased mortality (25% at 10 mg/kg/day), clinical signs (labored respiration and/or audible respiration, loose feces), and decreased body weight gain (3 and 10 mg/kg/day) during the dosing period.

Based on these results, the NOEL and LOEL for maternal toxicity were 1 and 3 mg/kg/day, respectively.

Developmental Toxicity

No compound-related anomalies were noted. However, altered growth (evidenced by decreased fetal body weight) and an increased number of dead fetuses were observed at 10 mg/kg/day. Consequently, the NOEL for developmental toxicity was 3 mg/kg/day; the LOEL was 10 mg/kg/day.

STUDY/REPORTING DEFICIENCIES

Food consumption data were not provided. However, the test material was administered by gavage, and therefore, this deficiency does not affect the interpretation of study results.

The historical control data on the incidence of dead fetuses/litter was not provided; these data would have been useful in confirming the compound-related deaths in pups.

E. CORE CLASSIFICATION: Minimum. This study satisfies guideline requirements [83-3b] for a developmental toxicity study in rabbits and is acceptable for regulatory purposes.

Maternal NOEL = 1 mg/kg/day
Maternal LOEL = 3 mg/kg/day based on clinical signs and decreased body weight gain

Developmental Toxicity NOEL = 3 mg/kg/day
Developmental Toxicity LOEL = 10 mg/kg/day based on decreased fetal body weight and an increased number of dead fetuses

F. RISK ASSESSMENT: Not applicable

TABLE 1. Incidence of Maternal Clinical Observations During Dosing Period[a]

Findings[b]	Dose Level (mg/kg/day) 0	1.0	3.0	10.0
Hypoactivity	0	0	2	1
Labored respiration	0	0	0	4
Audible respiration	0	0	3	7
Gasping	0	0	0	2
Abdominal breathing	0	0	0	1
Slow respiration	0	0	2	8
Loose feces	1	2	3	3
Blood on paperboard	0	0	0	1
Perioral wetness	0	2	3	1

[a]Data were extracted from study number 51-590, table 4.

[b]More than one clinical sign may be found in one animal.

[**]Significantly different from control ($p<0.01$)

TABLE 2. Body Weight Gain (g)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GDs 0-6)		Dosing Period (GDs 6-19)		Postdosing Period (GDs 19-29)		Entire Gestation Period (GDs 0-29)		Corrected Body Weight Change[b] (GDs 0-29)	
0	151.5	111.2[c]	154.2	106.6	111.5	125.4	417.1	198.2	-110.3	165.6
1.0	181.7	98.3	85.7	190.9	161.2	112.7	428.6	268.2	-145.8	219.5
3.0	128.4	87.2	29.7	94.0[**]	125.4	138.0	283.4	118.6	-188.5	155.0[*]
10.0	124.2	95.7	-159.7	288.1[*]	256.9	131.2	252.6	326.2	-248.3	207.9

[a]Data were extracted from study number 51-590, tables 3 and 7.

[b]Corrected body weight change = (Body weight on GD 29 - body weight on GD 0) - gravid uterus weight

[c]Mean S.D.

[*]Significantly different from control ($p<0.05$)

[**]Significantly different from control ($p<0.01$)

TABLE 3. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0		1.0		3.0		10.0	
No. animals assigned	16		16		16		16	
No. animals pregnant	14		15		15		14	
Pregnancy rate (%)	88		94		94		88	
Maternal wastage								
No. died/nonpregnant	0		0		0		0	
No. died/pregnant	1		0		0		4	
No. nonpregnant	2		1		1		2	
No. aborted/early delivery	0		2		0		1	
Gravid uterine weight (g)	527.5		574.4		508.9		500.8	
Litters w/live fetuses	14		13		15		9	
Total corpora lutea	155		137		160		98	(9)[b,c]
Corpora lutea/doe	11.1	2.0[b]	10.5	1.3	10.7	1.5	10.9	1.6
Total implantations	129		125		149		91	(9)[b,c]
Implantations/doe	9.2	2.4	9.6	1.3	9.9	2.0	10.1	1.9
Total live fetuses	120		117		143		81	(9)[b,c]
Live fetuses/doe	8.6	2.9	9.0	1.5	9.5	1.8	9.0	1.4
Total resorptions	8		4		5		4	
Early	8		4		4		4	
Late	0		0		1		0	
Resorptions/doe	0.6	0.9	0.3	0.5	0.3	1.0[d]	0.4	1.3[d]
Total dead fetuses	1		4		1		6	
Dead fetuses/doe	0.1	0.3	0.3	0.5	0.1	0.3	0.7	0.8[d]
Mean fetal weight (g)	40.6	3.4	40.8	4.0	40.2	4.0	35.5	7.6
Preimplantation loss (%)	17		9		7		7	
Postimplantation loss (%)[e]	7		6		4		11	
Sex ratio (% male)	48		57		46		46	

[a]Data were extracted from study number 51-590, Tables 2, 7, 8, and Appendix 3, Table 3.

[b]Mean S.D.

[c]Total number of litters included in calculation

[d]Excludes animals found dead or removed from study

[e]Calculated by the reviewers (but not analyzed) using individual data

[d]Significantly different from control ($p \leq 0.05$)

TABLE 4. Summary of Selected Fetal Malformations[a]

Findings[b]	Dose Level (mg/kg/day) 0	1.0	3.0	10.0
No. fetuses (litters) examined	120 (14)	117 (13)	143 (15)	81 (9)
External Examination				
Clubbed limb(s)	0	0	0	1
Scoliosis	0	0	0	1
Short tail	0	0	0	1
Gastroschisis	0	1	1	0
Rigid joint(s)	0	0	0	2 (1)
Umbilical hernia	0	0	1	0
Dome-shaped head	0	0	1	0
Total no. fetuses (litters) with any external malformation	0	1 (1)	3 (3)	3 (2)
Visceral Examination				
Short ureter	1	0	0	0
Lateral ventricle dilated, -tissue depressed	1	4 (1)	5 (4)	1
Missing azygous lung lobe	6 (5)	0	3 (3)	3 (3)
Gastroschisis	0	1	1	0
Umbilical hernia	0	0	1	0
Missing -kidney	0	0	0	1
-gall bladder	0	2 (1)	1	0
-ovary	0	1	0	0
-uterine horn	0	1	0	0
-urinary bladder	1	0	0	0
Total no. fetuses (litters) with any visceral malformation	8 (5)	7 (3)	11 (7)	4 (4)
Skeletal Examination				
Lateral scoliosis	2 (2)	0	0	2 (1)
Thoracic centra - fused	0	0	0	1
Thoracic arches - fused	0	0	0	1
Thoracic arches misshapen	0	0	0	1
All lumbar arches misshapen	0	0	0	1
Lumbar arches - fused	0	0	0	1
Lumbar centra - fused	0	0	0	1
Lumbar centrum fused to arch	0	0	0	1
Last thoracic centrum fused to first lumbar	0	0	0	1
Lateral rotation of lumbar vertebral segments	0	0	0	1
Extra lumbar arches #8 - unilateral	1	0	0	0
- bilateral	0	1	0	0
Extra lumbar centum #8	0	1	0	0
Fragmentation of sacral vertebral segments	0	0	0	1
Scrambled ossification sites at tip of tail	0	0	0	1
Rib (s)- fused	0	0	0	1
#11 missing	0	0	0	1
#12 missing	0	0	0	1
Extra rib #13 bent	0	0	0	1
Sternebrae duplicated	1	0	1	0
Total no. fetuses (litters) with any skeletal malformation	4 (3)	1	1	2 (1)
Total no. fetuses (litters) with any malformation	11 (6)	7 (3)	13 (7)	7 (5)

[a]Data were extracted from study number 51-590, Table 9.
[b]More than one type of anomalies may be found in one fetus.
[c]Significantly different from control ($p<0.5$) on a litter basis

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ATTACHMENT 1

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	Y/N	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available.
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	NO	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with an * are supplemental, may not be required for every study.

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FINAL

DATA EVALUATION REPORT

BARDAC 22

Study Type: Reproductive Toxicity

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer	Sanju Diwan, Ph.D.	Date 11/16/93
Independent Reviewer	Ila Lindstrom, DPH	Date 11/16/93
QA/QC Manager	Sharon Segal, Ph.D.	Date 11/16/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 291
Project Officer: Caroline Gordon

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Guideline Series 83-4: Reproductive Toxicity

EPA Reviewer: Robert Fricke, Ph.D. Signature: Robert F. Fricke
Review Section IV, Toxicology Branch II/HED Date: 17 Nov 93

EPA Section Head: Jess Rowland, M.S. Signature: Jess Rowland
Review Section IV, Toxicology Branch II/HED Date: 11/22/93

DATA EVALUATION REPORT

STUDY TYPE: Reproductive toxicity (rats); Guideline Series 83-4

EPA IDENTIFICATION NUMBERS

DP BARCODES: D188679, D189620 SUBMISSION NO.: S436173

P.C. CODE: 069149 CASWELL NO.: 331A

MRID NUMBER: 418045-01

TEST MATERIAL: BARDAC 22

SYNONYMS: Didecyldimethylammoniumchloride

SPONSOR: Lonza, Inc., Fair Lawn, NJ

STUDY NUMBER: 52-648

TESTING FACILITY: Bushy Run Research Center, Export, PA

TITLE OF REPORT: Two-Generation Reproduction Study in Sprague-Dawley (CD®) Rats with Didecyldimethylammoniumchloride Administered in the Diet

AUTHOR: T.L. Neeper-Bradley

REPORT ISSUED: February 1, 1991

CONCLUSIONS: In a two-generation four-litter reproduction study, CD® (Sprague-Dawley) rats were fed BARDAC 22 in the diet at dosage levels of 0, 300, 750, or 1500 ppm (during premating, for males 20, 50, or 103 mg/kg/day and for females 24, 61, or 122 mg/kg/day, respectively).

Parental NOEL = 750 ppm (56 mg/kg/day)
Parental LOEL = 1500 ppm (113 mg/kg/day) based on decreased body weight/body weight gain and food consumption

Reproductive NOEL = 750 ppm (56 mg/kg/day)
Reproductive LOEL = 1500 ppm (113 mg/kg/day) based on decreased mean pup body weight/weight gain during the postnatal period

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CORE CLASSIFICATION: Guideline. This study meets the guideline requirement [83-4] for a two-generation reproductive toxicity study in rats, and is acceptable for regulatory purposes.

A. MATERIALS

Test Compound

Purity:	80.8%
Description:	Viscous honey-colored liquid
Lot number:	B-1889
Dates Received:	August 13, 1987; November 24, 1987
Contaminants:	None reported
Storage:	At room temperature

Vehicle: None used; the test material was administered in the diet.

Test Animals

Species:	Rat
Strain:	Crl:CD® (SD) BR
Source:	Charles River Breeding Laboratories, Kingston, NY
Age:	Approximately 6 weeks at study initiation
Weight:	F_0 males--186-229 g at study initiation F_0 females--137-170 g at study initiation

B. STUDY DESIGN

This study was designed to assess the potential of BARDAC 22 to cause reproductive toxicity when administered continuously in the diet for two successive generations.

Mating: After 14 days of acclimatization followed by 70 days of dietary treatment, F_0 females were mated with males from the same group in a ratio of 1:1 until a plug or sperm was detected in a vaginal smear. After 7 days of mating, females failing to mate were mated with previously unsuccessful males from the same group. If after an additional 7 days females had still failed to mate, they were mated with yet another male from a previously unsuccessful pairing for a third 7 day period (for a maximum mating period of 21 days). For females failing to show evidence of successful mating after 21 days, the last scheduled mating day was considered GD 0. After weaning of the F_{1A} pups, F_0 females were rested for 10 days and then mated again. During the second mating, when possible, previously nonpregnant females and males failing to induce pregnancy were paired with other successfully mated animals. Females with no evidence of mating after 7 days were paired a second and a third time using the same procedure as in the F_{1A} mating.

Following 70 days of dietary treatment, F_1 animals were paired in a similar fashion as described above. Sibling matings were avoided.

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Animal husbandry: Food (Certified Ground Rodent Chow® #5002, Ralston-Purina Company, St. Louis, MO) and tap water were provided ad libitum and were analyzed for contaminants. Temperature and humidity data were recorded continuously (no details given), and a 12/12-hour light/dark cycle was maintained.

Group arrangement: Mated animals were distributed amongst four groups using a computer-generated randomization procedure based on body weight as follows:

Test Group	Dietary Level (ppm)	Number Assigned per Group F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	28	28	28	28
Low dose	300	28	28	28	28
Mid dose	750	28	28	28	28
High dose	1500	28	28	28	28

Diet preparation: The test material was administered in the diet for two consecutive generations. Test diets were prepared weekly and stored at room temperature. They were adjusted for purity; corrections were made for the loss of ethanol during preparation. The premix was prepared by mixing the test material with ground feed in a Hobart mixer for approximately one hour. The proper concentrations were achieved by diluting the premix or higher concentration diets with rodent chow and mixing for an additional 15 minutes. Stability and homogeneity of the test material in the diet were analyzed prior to study initiation. Concentration was analyzed weekly for the first four weeks of the study and then on samples of test diets from every fourth preparation.

Dosage rationale: The dosages were selected based on the results of a 2-week and a 90-day dietary studies in rats performed at Bushy Run Research Center. The references and results of these studies were not provided.

Observations: During the premating period, animals were examined twice daily for mortality and moribundity and once daily for clinical signs of toxicity. Body weight data were recorded weekly during premating and for females on days 0, 6, 15, and 20 of gestation and 0, 7, 14, and 21 of lactation. Male body weight data were recorded weekly for the remainder of the study. Food consumption data were recorded weekly and for females at three- to four-day intervals throughout gestation and from day 0 through 14 of lactation.

The following data were recorded for each litter:

- Number of live and dead pups
- Individual pup weight--at birth and on lactation days 4, 7, 14, 21, and 28
- Sex and gross abnormalities--daily

Uteri of apparently nonpregnant females were stained with potassium ferricyanide to detect early embryonic loss.

On day 4, pups were randomly culled to 4/sex/litter whenever possible; culled pups were examined externally, sacrificed and discarded. Any pups dying during lactation were necropsied to investigate cause of death. Twenty-eight male and 28 female F_{1B} pups were randomly selected as F_1 parental animals. All F_1 pups not selected for necropsy or for the F_1 parental group were examined for gross external abnormalities, euthanized and discarded. For both generations, the pups were weaned on day 21 postpartum. The weanlings, however, remained together as a litter until postpartum day 28, at which time they were either selected randomly for necropsy (10/sex/group), euthanized (remaining F_{1A} and F_{2A} pups), or selected to be parents for the F_2 generation (now referred to as F_1 adults).

Parental animals of both generations and 10 pups/sex/generation/group were sacrificed and necropsied after weaning on day 28. The tissues listed below were preserved in 10% neutral buffered formalin solution. Histopathology was conducted on these organs from the control and high-dose groups. Any of these organs or tissues showing gross alterations were also evaluated microscopically in the low- and mid-dose groups.

- Gross lesions
- Testes w/epididymes
- Seminal Vesicles
- Prostate glands
- Ovaries
- Uterus
- Vagina

Testes from adult males that did not sire a litter were also examined histologically.

Statistical analysis: The following analyses were conducted.

- Parametric data-- Levene's test for equal variances, ANOVA, and t-tests corrected by the Bonferroni method
- Nonparametric data-- Kruskal-Wallis test followed by the Mann-Whitney U-test
- Frequency data-- Fischer's exact test

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated January 11, 1991, was provided.
- A signed Statement of Compliance with FIFRA GLPs dated January 11 and 12, 1991, was provided.
- A signed Quality Assurance Statement, dated February 1, 1991, was provided.

- A signed FIFRA Flagging Statement, dated January 11, 1991, was provided.

C. RESULTS

Test Material Analysis: The mean measured concentrations for the three dose levels used in the study ranged from 95% to 109% of nominal values. Homogeneity analyses revealed concentrations from 95% to 114% of nominal values. Stability of the test material over 21 days in a closed polyethylene container at room temperature was 96%-107% of target; stability over 14 days in an open glass jar was found to be 96%-106% of target.

Systemic Toxicity

Mortality: No compound-related mortalities were observed in either sex or generation. Incidental deaths/moribund sacrifices are described below.

In the F_0 generation, at 1500 ppm, one male was euthanized after a cagin accident on study day 21. Necropsy revealed an oral/pharyngeal malocclusion, gas filled GI tract, traumatized head, perinasal encrustation, and brain hemorrhage. One male from the 750 ppm group was sacrificed moribund on study day 170. Necropsy revealed stained skin, hydronephrosis and kidney nodule, and dilatation or distension of the urinary bladder with calculus. One male from the 300 ppm group was euthanized on study day 146 because of trauma. Necropsy revealed staine skin, necrotic tail, increased size of the sub-mandibular lymph node, a a mass at bone joint.

There were no incidental deaths/moribund sacrifices in the F_1 generation

Clinical observations: No compound-related clinical signs were observed in either sex or generation.

Body weight: Compound-related effects in body weight and body weight gain were observed in both sexes and generations at 1500 ppm. Summaries of body weight and body weight gain data for selected intervals are presented in Tables 1 and 2. Detailed results are discussed below.

For F_0 males, body weight (Table 1) was significantly (≥5%; $p<0.05$) lowe at 1500 ppm during weeks 1-10 of premating, and during weeks 11 and 15 o mating (6%); and at 750 ppm during week 2 of premating (4%; $p<0.05$). Body weight gain (Table 2) for males at 1500 ppm was lower than control during weeks 0-2 and 4-6 of the premating period (≥17%; $p<0.01$) and week 25-26 postmating (≥45%; $p<0.01$). An incidental increase in body weight gain was seen in this dose group during weeks 15-16 and 26-27. At 750 ppm, it was also significantly reduced during weeks 0-2 and 4-6 of the premating period (≥9%; $p<0.01$) and during weeks 14-15 (33%; $p<0.05$) and 25-26 (60%; $p<0.01$) postmating. An incidental increase in body weight gain for these males was seen during weeks 15-16 (42%; $p<0.05$) and 20-21 (120%; $p<0.01$).

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For F_0 females, body weight at 1500 ppm (Table 1) was significantly (≥4%; p≤0.05) lower than control on weeks 1, 2, 5, 6, 7 and 9 of the premating period; days 0, 6, and 15 of the first gestation (≥5% p<0.05); day 7 of the first lactation (4%; p<0.01); and day 0 (7%; p<0.05) of the second lactation. Body weight gain (Table 2) at 1500 ppm was lower (≥31%; p<0.01) compared to control during weeks 0-1 and 4-5; an incidental increase in body weight gain was noted at 750 ppm during weeks 5-6 (62%; p<0.05). Sporadic decreases and increases in body weight gain in all three dosage groups observed during the two gestation and lactation periods were considered incidental (data not shown).

For F_1 males, body weight (Table 1) at 1500 ppm decreased (≥6%; p≤0.05) during weeks 0-16 (premating, mating, and postmating). Significant (≥6%; p<0.05) increases in body weight were observed during weeks 17-27 (with the exception of weeks 22 and 26) at 750 ppm and during weeks 6-10 of premating (≥5%; p<0.05), and weeks 11-23 of mating and postmating (≥6%; p<0.05) at 300 ppm. Body weight gain (Table 2) at 1500 ppm was reduced significantly (17%; p<0.01) during weeks 0-1 (premating) and 12-13 (41%; p<0.01), but on week 20, it was significantly higher than control (>200%; p<0.01). Other incidental increases in body weight gain occurred at 750 ppm on weeks 6-7 and 15-17, and at 300 ppm on weeks 5-6.

For F_1 females, body weight (Table 1) at 1500 ppm was significantly (≥7%; p<0.05) lower than control on weeks 0-10 of the premating period; days 0 and 6 of the first gestation (≥7%; p<0.05); days 0 and 15 of the second gestation (≥8%; p<0.05); and day 0 of the first and second lactation periods (≥8%; p<0.05). Body weight gains (Table 2) of these females were significantly lower than control on weeks 0-1 (23%; p<0.01) and 8-9 (50%; p<0.01) during premating. With the exception of a 20% decrease in body weight gain at 750 ppm during the first week of premating, the body weight gains were comparable with control at 750 and 300 ppm. Sporadic decreases and increases in body weight gains in all three dosage groups observed during the two gestation and lactation periods were considered incidental.

Food consumption: Compound-related effects were observed in food consumption during premating at 1500 ppm in both sexes and generations. Sporadic changes in food consumption at 750 and 300 ppm were not considered to be compound related. A summary of food consumption data for the selected intervals is presented in Table 3.

For F_0 males, a significant decrease (≥6%) in food consumption was noted at 1500 ppm during weeks 0-10 of premating (except for weeks 7-8) and during weeks 17-19 and 25-26 postmating. Incidental decreases (≥5%) in food consumption were noted at 750 ppm during weeks 0-2 and 18-19.

For F_0 females, food consumption decreased (≥7%) during week 0-1 and 5-6 of premating. It was comparable among all dose groups during gestation and lactation (except for an incidental increase at 300 ppm during weeks 0-4 of the second gestation).

For F_1 males, a significant decrease (≥7%) in food consumption was noted at the 1500 ppm during weeks 0-7 of premating. Significant increases (≥6%) in food consumption noted at 750 ppm during weeks 13-14, 15-17, and 20-21 postmating and at 300 ppm during weeks 4-18 of premating, mating,

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and postmating (except for weeks 16-17) were not considered to be compound related.

For F_1 females, food consumption was significantly (≥7%) decreased durin premating weeks ^-10, except for weeks 6-8. It was comparable among al dose groups during gestation and lactation periods.

Test material consumption: The mean test material consumption (mg/kg/day) for both sexes during premating was as follows:

Sex	Dosage Groups (ppm) 300	750	1500
Males			
F_0	21.1	52.6	104.2
F_1	19.4	48.2	101.0
Females			
F_0	25.3	62.6	124.9
F_1	23.2	58.7	119.8

Gross/histopathology: No compound-related gross or histologic findings were observed in either sex or generation.

Reproductive Toxicity: Compound-related reproductive effects were observed at 1500 ppm and consisted of decreased body weight/weight gain in pups. Summaries of these effects are presented in Tables 4-7. Detailed results are discussed below.

For F_{1A} and F_{1B} pups, body weights (Tables 4 and 5, respectively) were significantly reduced compared to control on days 21-28. Body weight gain (Tables 4 and 5, respectively) for these pups were ≥14% (F_{1A}) and ≥16% (F_{1B}) lower than control on days 21-28 and 14-28, respectively.

For F_{2A} and F_{2B} pups, body weights (Tables 6 and 7, respectively) were significantly reduced on days 28 and 14-28, respectively. Body weight gains (Tables 6 and 7, respectively) for these pups were ≥11% lower than control during days 14 (males only) to 28 and 4-28 (except for days 7-14), respectively. The significant increase in sex ratio in F_{2B} pups at 1500 and 750 ppm were not considered to be compound related.

No compound-related effects were observed in the clinical or pathologica findings in pups from any litter or generation (data not shown).

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D. REVIEWERS' DISCUSSION/CONCLUSIONS

Test Material Analyses: The purity of the test compound in the test diet was confirmed. Concentration, homogeneity, and stability analyses revealed mean values within ±14% of nominal values.

Parental Toxicity: Compound-related toxicity was observed at 1500 ppm in both sexes and generations and consisted of significant reductions in body weight/weight gain, and food consumption. No compound-related effects were seen in mortality, clinical signs, or gross or microscopic observations.

Based on these results, the NOEL and LOEL for parental toxicity were 750 and 1500 ppm, respectively.

Reproductive Toxicity: Compound-related reproductive toxicity was observed at 1500 ppm. It was manifested as decreased pup body weight and weight gain during later part of the postnatal period. This decrease occurred consistently in both generations and all matings and was most likely a consequence of the pups starting to eat the diet on their own. However, owing to the potential consequences of altered growth in such organ systems as the immune and nervous systems, which are not fully developed until adulthood, these effects are considered to be biologically significant. No compound-related effects were observed for any other reproductive parameter.

Based on these results, the NOEL and LOEL for reproduc ive toxicity were 750 and 1500 ppm, respectively.

Parental toxicity NOEL = 750 ppm (56 mg/kg/day)
Parental toxicity LOEL = 1500 ppm (113 mg/kg/day) based on decreased body weight/weight gain, and food consumption)

Reproductive toxicity NOEL = 750 ppm (56 mg/kg/day)
Reproductive toxicity LOEL = 1500 ppm (113 mg/kg/day) based on decreased pup body weight/weight gain)

E. CORE CLASSIFICATION: Guideline. This study meets the guideline requirements (83-4) for a two-generation reproductive toxicity study in rats, and is acceptable for regulatory purposes.

F. RISK ASSESSMENT: Not applicable

Table 1. Body Weight (g ± S.D.) During the Premating Period for Rats Fed Didecyldimethylammoniumchloride for Two Successive Generations[a]

Study Weeks	Dietary Level (ppm): 0		300		750		1500	
F_0 Males								
1	268.6	14.7	267.8	13.9	262.9	14.3	254.9	13.2**
3	359.4	21.3	355.6	23.0	351.4	21.5	334.7	21.9**
5	426.2	28.8	421.2	30.4	415.6	30.0	395.9	24.7**
7	470.5	35.0	462.9	35.4	461.4	37.0	439.7	29.6**
9	510.6	41.7	498.7	41.6	500.4	43.4	475.8	30.9**
F_0 Females								
1	182.3	10.3	183.2	10.6	180.8	10.5	174.3	10.7*
3	222.6	15.4	217.0	17.9	220.0	18.6	212.6	16.1
5	250.7	18.5	248.3	22.3	246.9	24.9	232.6	18.5**
7	268.6	24.6	267.0	22.5	259.8	28.6	251.5	20.5*
9	282.5	27.4	283.2	24.0	285.5	34.0	264.2	21.5*
F_1 Males								
1	347.1	28.9	361.4	26.1	356.0	28.3	301.4	28.7**
3	422.7	28.6	439.5	32.2	434.2	31.8	375.3	30.7**
5	476.2	32.6	499.2	37.8	489.6	38.5	427.3	33.1**
7	513.5	35.3	542.3	43.1*	534.2	43.4	467.3	36.9**
9	542.9	44.4	580.3	48.8**	566.8	48.7	499.3	40.5**
F_1 Females								
1	221.5	20.8	232.0	17.2	223.8	21.9	197.1	19.1**
3	255.8	20.6	267.5	22.1	257.6	22.8	231.6	22.1**
5	279.4	26.5	289.2	25.3	281.9	27.2	253.1	24.6**
7	291.7	23.6	305.2	27.9	297.3	25.9	268.5	27.4**
9	305.2	27.5	318.3	28.2	311.3	31.0	279.2	26.6**

[a]Data were extracted from Study No. 52-648, Tables 4, 6, 33 and 35.

*Significantly different from control ($p<0.05$)

**Significantly different from control ($p<0.01$)

Table 2. Body Weight Gain (g ± S.D.) During the Premating Period for Rats Fed Didecyldimethylammoniumchloride for Two Successive Generations[a]

Study Weeks	Dietary Level (ppm) 0		300		750		1500	
F_0 Males								
1- 2	49.7	5.6	47.5	8.3	43.3	4.9**	41.1	5.9**
3- 4	33.4	8.1	33.0	7.3	35.8	6.3	33.6	6.7
5- 6	24.2	5.0	24.0	5.4	23.1	6.8	19.4	5.6**
7- 8	16.2	5.7	15.9	7.3	19.9	5.2	16.8	6.8
9-10	14.3	8.0	16.2	5.5	13.7	6.6	16.0	7.3
F_0 Females								
1- 2	21.4	5.1	18.3	5.0	18.8	6.1	18.5	6.6
3- 4	13.6	6.2	15.6	4.7	16.0	7.0	12.3	5.7
5- 6	7.6	6.7	9.6	5.4	12.6	6.7*	9.4	5.8
7- 8	5.0	4.8	3.3	15.9	6.6	5.2	5.6	4.9
9-10	3.3	5.9	5.0	6.0	4.9	3.8	5.3	3.8
F_1 Males								
1- 2	42.6	5.9	44.6	8.2	40.9	6.2	38.9	7.1
3- 4	28.0	5.9	30.8	5.1	30.0	7.3	27.9	5.9
5- 6	21.6	5.9	25.9	5.3*	21.6	7.5	22.1	6.4
7- 8	14.9	8.7	17.6	6.8	18.0	4.8	16.6	6.6
9-10	8.9	18.2	7.4	7.0	8.6	6.4	9.7	6.8
F_1 Females								
1- 2	18.2	7.2	19.7	7.8	17.1	5.2	16.1	5.8
3- 4	10.9	5.8	10.5	6.0	13.2	7.0	10.0	6.3
5- 6	6.2	7.6	9.4	5.8	7.0	4.7	6.4	6.5
7- 8	4.1	6.2	5.2	5.9	5.5	6.6	6.1	6.5
9-10	5.4	7.9	6.1	6.0	4.9	6.5	5.2	4.4

[a]Data were extracted from Study No. 52-648, pp. 39, 42, 85, and 88.

*Significantly different from control ($p<0.05$)

**Significantly different from control ($p<0.01$)

Table 3. Food Consumption (g/animal/day ± S.D.) During the Premating Period for Rats Fed Didecyldimethylammoniumchloride for Two Successive Generations[a]

Study Weeks	Dietary Level (ppm) 0		300		750		1500	
F_0 Males								
1- 2	27.2	1.8	24.3	2.2	25.7	1.7*	25.3	2.3**
3- 4	27.3	2.2	26.8	2.1	27.0	2.0	25.8	2.2*
5- 6	28.1	2.3	27.4	2.4	26.6	2.5	24.9	2.4**
7- 8	27.6	2.6	26.5	2.3	27.3	1.9	26.1	2.3
9-10	28.0	2.5	27.7	2.7	26.5	2.3	26.1	3.0*
F_0 Females								
1- 2	20.1	1.8	19.5	1.8	19.3	1.8	18.8	1.8
3- 4	21.0	2.2	20.1	2.3	20.2	2.3	18.9	1.8**
5- 6	20.1	2.1	19.8	2.0	19.8	2.2	18.6	1.9*
7- 8	19.6	2.1	19.9	2.2	20.0	2.3	18.8	1.7
9-10	18.9	2.2	18.8	3.1	19.1	2.0	18.3	1.8
F_1 Males								
1- 2	29.1	1.8	29.7	2.4	29.2	2.4	26.5	2.0**
3- 4	29.0	2.1	30.3	2.6	30.1	2.2	27.1	2.0**
5- 6	29.0	2.1	30.8	2.5*	29.8	3.2	26.7	1.9**
7- 8	28.8	2.7	30.6	2.9*	29.9	2.8	27.4	2.4
9-10	27.8	5.1	30.8	2.8**	29.0	2.8	26.4	2.3
F_1 Females								
1- 2	21.2	2.0	21.8	2.0	20.8	1.9	18.8	1.8**
3- 4	21.4	2.1	21.7	1.7	21.6	2.2	19.8	2.6*
5- 6	21.1	2.4	21.6	1.9	21.0	1.7	19.3	1.7**
7- 8	20.0	2.7	20.8	1.9	21.2	2.0	19.6	2.0
9-10	20.3	3.0	21.1	1.8	20.4	2.2	18.5	1.9**

[a]Data were extracted from Study No. 52-448, pp. 43, 44, 89, and 92.

*Significantly different from control ($p<0.05$)

**Significantly different from control ($p<0.01$)

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Table 4. Effects of Dietary Administration of Didecyldimethylammoniumchloride on F_{1A} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary level (ppm)			
	0	300	750	1500
No. paired	28	28	28	27[b]
No. mating (% parents)	27	28	28	27[b]
Mating index (%; females)[c]	96	100	100	100
Mating index (%; males)[c]	96	100	100	100
Fertility index (%; females)[d]	82	86	71	96
Fertility index (%; males)[d]	82	86	71	96
Gestation index (%)[e]	100	100	100	100
Gestation length (days)	22.2	22.2	21.9	22.1
No. females with livebirth pups	22	24	20	26
Total no. live pups				
Day 0	286	324	277	380
Day 4 postcull	282	317	267	376
Day 21	169	191	158	207
Mean no. live pups/litter				
Day 0	13.0	13.5	13.9	14.6
Day 4 postcull	12.8	13.2	13.4	14.5
Day 21	7.7	8.0	7.9	8.0
Live birth index (%)[f]	100	99	99	99
Viability index (%)[g]	99	98	97	99
Lactation index (%)[h]	100	100	95	99
Mean pup body weight (g)				
Day 0	7.1	7.3	7.1	7.0
Day 7	16.9	17.2	17.1	16.3
Day 14	34.3	35.0	35.8	33.5
Day 21	59.2	58.2	56.8	50.9**
Day 28	93.3	94.4	95.9	83.6**
Mean pup body weight gain (g)				
Day 1-4	3.3	3.5	3.4	3.0
Day 7-14	17.8	18.8	18.7	17.2
Day 14-21	21.0	22.2	21.0	17.4**
Day 21-28	38.0	36.2	39.1	32.8**
Sex ratio (% males day 0)	52	54	49	48

[a]Data were extracted from Study No. 52-648, pp. 48, and 53-59.

[b]One male died prior to the mating period and, as no pairing switches occurred in the F_{0A} breed, one female was not mated.

[c]Mating index: (No. of plug-/sperm-positive females/No. of females paired) x 100; (No. males impregnating females/No. of males paired) x 100

[d]Fertility index: (No. of females pregnant/No. of plug-/sperm-positive females) x 100; (No. of males siring litters/No. of males impregnating females) x 100

[e]Gestation index: % of females with live litters/No. of pregnant females

[f]Live birth index: No. of pups live at birth/Total No. of pups born

[g]Viability index: No. of pups surviving four days/No. of pups live at birth

[h]Lactation index: Percentage of pups surviving 21 days/Total No. of live pups on day 4 postcull

**Significantly different from control ($p<0.01$)

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Table 5. Effects of Dietary Administration of Didecyldimethylammoniumchloride on F_{1b} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary Level (ppm) 0	300	750	1500
No. paired (females/males)	28/28	28/27[b]	28/28	28/27[b]
No. matings (females/males)	27/26	27/27	28/28	27/27
Mating index (%; females)[c]	96	96	100	96
Mating index (%; males)[c]	93	100	100	100
Fertility index (%; females)[d]	74	74	64	85
Fertility index (%); males)[d]	73	74	64	85
Gestation index (%)[e]	100	100	100	100
Gestation length (days)	22.1	22.1	22.0	22.3
No. females with liveborn pups	20	20	18	23
Total no. live pups				
Day 0	266	295	227	335
Day 4 precull	258	288	223	324
Day 21	149	160	126	183
Mean no. live pups/litter				
Day 0	13.3 (20)	14.8	12.6 (18)	14.6
Day 4 precull	13.6 (19)	14.4	13.1 (17)	14.1
Day 21	7.8 (19)	8.0	7.4 (17)	8.0
Live birth index (%)[f]	95	99	95	99
Viability index (%)[g]	93	98	93	97
Lactation index (%)[h]	99	100	99	99
Mean pup body weight (g)				
Day 1	7.2	7.0	7.1	7.1
Day 7	16.7	16.1	17.2	16.0
Day 14	34.1	33.8	34.9	32.6
Day 21	51.9	51.4	53.3	47.6**
Day 28	87.4	86.3	89.3	74.9**
Mean pup body weight gain (g)				
Day 1-4	3.3	3.1	3.6	3.2
Day 7-14	17.4	17.7	17.8	16.6
Day 14-21	17.8	17.6	18.4	15.1**
Day 21-28	35.5	34.9	35.9	27.3**
Sex ratio (% males day 0)	48	51	53	54

[a]Data were extracted from Study No. 52-648, pp. 60, and 62-72.

[b]One F_0 male in the 1500 ppm group died prior to the F_{1b} mating period and one F_0 male in the 300 ppm group died prior to the F_{1b} mating period. The unpaired females in each group were paired with a previously unsuccessful male in the same group.

[c]Mating index: (No. of plug-/sperm-positive females/No. of females paired) x 100; (No. of males impregnating females/No. of males paired) x 100

[d]Fertility index: (No. of females pregnant/No. of plug-/sperm-positive females) x 100; (No. of males siring litters/No. of males impregnating females) x 100

[e]Gestation index: No. of females with live litters/ No. of pregnant females

[f]Live birth index: No. of pups live at birth/Total No. of pups born

[g]Viability index: No. of pups surviving four days/No. of pups live at birth

[h]Lactation index: Percentage of pups surviving 21 days/Total No. of live pups on day 4 postcull

**Significantly different from control ($p<0.01$)

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Table 6. Effects of Dietary Administration of Didecyldimethylammoniumchloride on F_{2A} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary Level (ppm) 0	300	750	1500
No. paired	28	28	28	28
No. matings (F_1 parents)	28	27	27	28
Mating index (%; females)[b]	100	96	96	100
Mating index (%; males)[b]	100	96	96	100
Fertility index (%; females)[c]	71	74	93	93
Fertility index (%; males)[c]	71	74	93	93
Gestation index (%)[d]	100	100	100	100
Gestation length (days)	22.1	22.1	22.0	22.1
No. females with liveborn pups	20	20	25	26
Total no. live pups				
Day 0	247	279	351	341
Day 4 precull	237	273	342	334
Day 21	142	154	196	208
Mean no. live pups/litter				
Day 0	12.4 (20)	14.0	14.0	13.1
Day 4 precull	12.5 (19)	13.7	13.7	12.9
Day 21	7.5 (19)	7.7	7.8	8.0
Live birth index (%)[e]	96	98	99	97
Viability index (%)[f]	92	98	98	98
Lactation index (%)[g]	99	99	99	100
Mean pup body weight (g)				
Day 1	6.9	7.2	6.8	6.9
Day 7	16.2	17.2	16.3	16.0
Day 14	32.0	34.6*	32.9	31.5
Day 21	51.1	54.0	52.3	48.6
Day 28	87.5	90.4	84.6	78.8**
Mean pup body weight gain (g)				
Day 1-4	3.3	3.3	3.3	3.3
Day 7-14	15.7	17.4*	16.7	15.5
Day 14-21	19.2	19.4	19.4	17.1*
Day 21-28	36.2	36.4	34.3	30.2**
Sex ratio (% males day 0)	55	48	51	49

[a]Data were extracted from Study No. 52-648, pp. 94, 99-105.

[b]Mating index: (No. of plug+/sperm-positive females/No. of females paired) x 100; (No. of males impregnating females/No. of males paired) x 100

[c]Fertility index: (No. of females pregnant/No. of plug+/sperm-positive females) x 100; (No. of males siring litters/No. of males impregnating females) x 100

[d]Gestation index: No. of females with live litters/ No. of pregnant females

[e]Live birth index: No. of pups live at birth/Total No. of pups born

[f]Viability index: No. of pups surviving four days/No. of pups live at birth

[g]Lactation index: Percentage of pups surviving 21 days/Total no. of live pups on day 4 postcull

*Significantly different from control (p=0.05)

**Significantly different from control (p=0.01)

Table 7. Effects of Dietary Administration of Didecyldimethylammoniumchloride on F_{1B} Reproductive Parameters, Offspring Survival, and Pup Body Weight[a]

Parameter	Dietary Level (ppm) 0	300	750	1500
No. paired	28	28	28	28
No. matings (females/males)	28/28	25/24	27/27	28/28
Mating index (%; females)[b]	100	86	96	100
Mating index (%; males)[b]	100	89	96	100
Fertility index (%; females)[c]	68	64	78	93
Fertility index (%; males)[c]	68	63	78	93
Gestation index (%)[d]	100	100	100	100
Gestation length (days)	22.3	22.1	22.0	22.2
No. females with liveborn pups	19	16	21	26
Total no. live pups				
Day 0	226	199	284	342
Day 4 precull	223	195	277	338
Day 21	143	117	165	196
Mean no. live pups/litter				
Day 0	11.9	12.4	13.5	13.2
Day 4 precull	11.7	12.2	13.2	13.0
Day 21	7.5	7.3	7.9	7.5
Live birth index (%)[e]	99	98	100	99
Viability index (%)[f]	99	98	97	99
Lactation index (%)[g]	100	99	99	97
Mean pup body weight (g)				
Day 1	7.2	7.3	7.0	7.0
Day 7	16.9	17.1	16.6	16.1
Day 14	34.1	34.7	33.7	32.6
Day 21	54.8	55.7	54.6	50.5**
Day 28	91.5	92.2	92.2	82.5**
Mean pup body weight gain (g)				
Day 1- 4	3.4	3.5	3.3	3.4
Day 7-14	17.3	17.6	17.1	16.5
Day 14-21	20.7	21.0	20.9	17.6**
Day 21-28	36.8	36.5	37.7	32.5**
Sex ratio (% males day 0)	44	50	56**	54*

[a]Data were extracted from Study No. 52-648, pp. 106, 112-118.

[b]Mating index: (No. of plug-/sperm-positive females/No. of females paired) x 100; (No. of males impregnating females/No. of males paired) x 100

[c]Fertility index: (No. of females pregnant/No. of plug-/sperm-positive females) x 100; (No. of males siring litters/No. of males impregnating females) x 100

[d]Gestation index: No. of females with live litters/ No. of pregnant females

[e]Live birth index: No. of pups live at birth/Total No. of pups born

[f]Viability index: No. of pups surviving four days/No. of pups live at birth

[g]Lactation index: Percentage of pups surviving 21 days/Total No. of live pups on day 4 postcull

**Significantly different from control ($p \leq 0.01$)

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DATA EVALUATION REPORT

BARDAC 22

Study Type: Mutagenicity: In Vivo Cytogenetic Assay with Rats

Prepared for:

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

Prepared by:

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

Principal Reviewer Lynne T. Haber, Ph.D. Date 11/12/93

Independent Reviewer Nancy E. McCarroll, B.S. Date 11/12/93

QA/QC Manager Sharon Segal, Ph.D. Date 11/12/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 295
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY IN VIVO MAMMALIAN CYTOGENETICS

MUTAGENICITY STUDIES

EPA Reviewer: Robert Fricke, Ph.D. Signature: Robert F. Fricke
Review Section IV, Date: 11 Nov 93
Toxicology Branch II/HED (H7509C)
EPA Section Head: Jess Rowland, M.S. Signature: Jess Rowland
Review Section IV
Toxicology Branch II/HED (H7509C) Date: 11/19/93

DATA EVALUATION REPORT

CHEMICAL: BARDAC 22

DP BARCODES: D188679, D189620

PC CODE: 069149

SUBMISSION NUMBERS: S436173

CASWELL NUMBER: 331A

STUDY TYPE: Mutagenicity: In vivo chromosome aberration in rat bone marrow cells.

MRID Number: 407058-02

SYNONYMS/CAS No.: Didecyldimethylammonium chloride

SPONSOR: Lonza AG, Basle, Switzerland and Fair Lawn, NJ

TESTING FACILITY: Huntingdon Research Centre, Ltd., Huntingdon, England

TITLE OF REPORT: Analysis of Metaphase Chromosomes Obtained from Bone Marrow of Rats Treated with P0151

AUTHORS: Allen, J.A., Proudlock, R.J., and Brooker, P.C.

STUDY NUMBER: LZA 24/8761

REPORT ISSUED: April 1, 1987

CONCLUSIONS--EXECUTIVE SUMMARY: Reported to be negative in the rat bone marrow cytogenetics assay conducted with a single oral gavage administration of 600 mg/kg P0151 and harvests 6, 24, and 48 hours postexposure. Although P0151 treatment resulted in overt clinical signs of toxicity and no structural chromosome aberrations were observed at any harvest time, the percentage of

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cells with aberrations and the number of aberrations per cell induced by the positive control (cyclophosphamide at 40 mg/kg) were much lower than expected.[1] Therefore, the sensitivity of the assay to detect a weak clastogen is uncertain.

CORE CLASSIFICATION: Unacceptable. This study does not satisfy Guideline requirement (§84-2) for genetic effects Category II, Structural Chromosomal Aberrations and is unacceptable for regulatory purposes.

A. MATERIALS:

1. Test Material: P0151

Description: Yellowish liquid
Identification number: Batch number: E 06130085
Purity: 50.3% (The test material was a 50% solution of the active ingredient, Bardac 22)
Receipt date: Not reported
Stability: Not reported
Contaminants: Not reported
Solvent used: Sterile distilled water (DH_2O)
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions were prepared immediately prior to use. The report did not indicate whether purity adjustments were made or whether analytical determinations on representative dosing solutions were performed.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: DH_2O/20 mL/kg/oral gavage

Positive/final dose(s)/route of administration: Cyclophosphamide (?) was prepared in sterile 0.9% saline and was administered once by intraperitoneal injection at a dose of 40 mg/kg.

3. Test Compound:

Route of administration: Oral gavage

Volume of test substance administered: 20 mL/kg

[1] Gupta, P., Brem, R.J., Dubnaleve, J. (1975). Relationship between experimental results in mammals and man. I. Cytogenetic analysis of bone marrow injury induced by a single dose of cyclophosphamide. Mutat Res 31 247-254.

Dose levels used:

(a) Acute dose range-finding study: 200, 400, 600, 800, and 1000 mg/kg (2 males and 2 females/treatment group) were tested i phase 1, and 600, 800, 1000, and 1200 mg/kg (5 males and 5 females/treatment group) were tested in phase 2.

(b) Cytogenetics assay: 600 mg/kg (5 males and 5 females/treatment group/sacrifice time)

4. Test Animals:

(a) Species: Rat Strain: SPF CD Age: Not reported
Weight: 45-50 g at transmittal (males and females)
Sex: Males and females Source: Charles River UK Ltd., Margate, England.

(b) Number of animals used per dose:

- Treatment group: 15 males 15 females
- Positive control: 5 males 5 females
- Vehicle control: 15 males 15 females

Note: A secondary dose group of one male and one female receivin the high dose was included for use in case of morrality in the primary high-dose group. One high-dose female in the secondary dosing group was used in the study.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

Test compound
Dosing: X once ____ twice (24 hours apart)
____ other (describe):

Sampling (after last dose): X 6 hours ____ 12 hours
X 24 hours X 48 hours ____ 72 (mark all that are appropriate)
____ other (describe):

Negative and/or vehicle control
Dosing: X once ____ twice (24 hours apart)
____ other (describe):

Sampling (after last dose): _x_ 6 hours _____ 12 hours
_x_ 24 hours _x_ 48 hours _____ 72 (mark all that are appropriate)
_____ other (describe):

Positive control
Dosing: _x_ once _____ twice (24 hours apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hours _____ 12 hours
_x_ 24 hours _____ 48 hours _____ 72 (mark all that are appropriate)
_____ other (describe):

Administration of spindle inhibitor

Inhibitor used/dose: Colchicine/4 mg/kg

Administration time: Two hours prior to sacrifice

Route of administration _x_ i.p. _____ other (describe)

2. Tissues and Cells Examined:

_x_ bone marrow _____ other (list):

Number of cells per animal, per treatment group examined: 50.

Number of cells per animal, per control group examined: 50.

3. Details of Cell Harvest and Slide Preparation: Animals in the treatment and vehicle control groups were sacrificed by cervical dislocation at 6, 24, and 48 hours postexposure to the appropriate dose of the test material or vehicle. Animals in the positive control group were sacrificed 24 hours posttreatment. Bone marrow was flushed from both femurs using Hank's balanced salt solution. Collected marrow cells were centrifuged, resuspended in 0.56% KCl, incubated at room temperature and recentrifuged; the supernatants were discarded. Cell pellets were fixed overnight in methanol:acetic acid (3:1 at 4°C, dropped onto slides, air dried, stained with Giemsa, mounted and coded.

4. Evaluation Criteria: No criteria were provided to evaluate assay validity, a positive response, or the biological significance of the findings.

5. Statistical Methods: The percentage of cells with structural aberrations was evaluated using Wilcoxon's sum of ranks test at $p<0.05$ and $p<0.001$.

C. REPORTED RESULTS:

1. Preliminary Toxicity Assay: The first phase of toxicity testing wa conducted with 200, 400, 600, 800, or 1000 mg/kg administered by or gavage to two animals/sex/dose. Deaths were reported in animals exposed to 1000 mg/kg (one female), 800 mg/kg (both males and one female) and 600 mg/kg (one female). Numerous clinical signs of toxicity, including piloerection, hunched posture, decreased respir tory rate, lethargy, ptosis, and pallor of extremities were reporte at levels ≥600 mg/kg and persisted for 72 hours. Clinical signs in the 200- and 400-mg/kg groups were limited to slight piloerection a hunched posture, which were reversed by 45 hours.

   Based on these results, a second toxicity assay was conducted with five animals/sex/dose receiving doses of 600, 800, 1000, o. 1200 mg/ kg. Three males and three females in the high-dose group died (one each sex at 24, 29, and 45 hours). Additional deaths were two male and one female treated with 1000 mg/kg (at 29, 45, and 24 hours, respectively), and one female treated with 800 mg/kg at 24 hours. Clinical signs at 600 mg/kg were slight piloerection, hunched postu lethargy and diarrhea. These effects were also observed at higher doses with severity ranging from slight to severe. Decreased respiratory rate, ptosis, pallor of extremities, bloated abdomen, a walking on toes were also noted at ≥800 mg/kg. Based on the combine results of the toxicity assays, the cytogenetics assay was conducted with 600 mg/kg.

2. Cytogenetic Assay: Groups of male and female rats were dosed with 600 mg/kg P0151, or the vehicle (DH_2O) or positive (40 mg/kg CP) controls. One treatment-group female died at 28.5 hours and was replaced by the secondary-group female. Clinical signs of toxicity were in agreement with those reported in the preliminary toxicity tests, and included moderate piloerection, moderate-to-severe hunche posture, lethargy, decreased respiratory rate, ptosis, diarrhea, and pallor of the extremities.

   Results from the bone marrow cytogenetic phase of the assay are presented in Table 1. As shown, no aberrations were observed in cel harvested from male or female rats 6, 24, or 48 hours postexposure t 600 mg/kg P0151 or the vehicle. In the positive control group, the combined number of aberrations per cell (0.09) was increased and the combined percentage of cells with aberrations (5.6) was significantl (p<0.001) higher than the control. However, Goetz et al. (1975)[2] found that intraperitoneal administration of 40 mg/kg CP to Wistar rats resulted in 0.64 aberrations/cell and ≈29.6% cells with aberrations. Based on our reviewers' experience with this test

[2] Goetz, P.; Brem, R.J., Dennelova, J. (1975) Mutat. Res. 31:247-250.

system, which is supported by the literature, we consider the response induced by 40 mg/kg CP in the current study to be inadequate.

Nevertheless, the study authors concluded that P0151 was not clastogenic in this in vivo rat bone marrow cytogenetic assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that no conclusion can be drawn from the testing of 600 mg/kg P0151 in the rat bone marrow cytogenetic assay with harvests at 6, 24, and 48 hours. Although toxic levels were reached and no chromosome aberrations were observed, the weak response induced by the positive control (40 mg/kg CP) raised concerns regarding the sensitivity of the test system to detect weak clastogens. We conclude, therefore, that the study was unacceptable.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement signed and dated April 1, 1987 indicated that an in-life inspection was conducted on this type of assay at or about the time of the study under review. The report was, however, audited by the quality assurance unit.)

CORE CLASSIFICATION: Unacceptable. This study does not satisfy the data Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosomal Aberrations and is unacceptable for regulatory purposes.

TABLE 1. Results of the Rat Bone Marrow Cytogenetics Assay with P0151

Treatment/Dose	Dose	Exposure Time[a] (Hours)	No. of Animals Analyzed	No. of Metaphases Analyzed	Total Number of Aberrations	Number of Aberrations/ Cell	% Cells with Aberrations[b]	Biologically Significant Aberrations Total No./Type
Vehicle Control								
Distilled water	20 mL/kg	6	5 M	250	0	0.00	0.0	--
		6	5 F	250	0	0.00	0.0	--
		24	5 M	250	0	0.00	0.0	--
		24	5 F	250	0	0.00	0.0	--
		48	5 M	250	0	0.00	0.0	--
		48	5 F	250	0	0.00	0.0	--
Positive Control								
Cyclophosphamide	40 mg/kg	24	5 M	250	25	[illegible]	NR	1BMF; 3I; 9BM; 2R; 9AF; 1F
		24	5 F	250	21	0.08 (0.09)[c]	NR (5.6*)	2BMF; 3I; 7BM; 6AF; 2GT; 1F
Test Material								
P0151	600 mg/kg	6	5 M	250	0	0.00	0.0	--
		6	5 F	250	0	0.00	0.0	--
		24	5 M	250	0	0.00	0.0	--
		24	5 F	250	0	0.00	0.0	--
		48	5 M	250	0	0.00	0.0	--
		48	5 F	250	0	0.00	0.0	--

[a]Time after compound exposure by oral gavage

[b]Excluding gaps

[c]Values in () are the combined average for the male and female dosing groups.

Abbreviations used:
BMF = Chromatid break with fragment
I = Interchange
BM = Single minute
F = Pulverised metaphase, counted as 1 aberration
R = Ring chromosome
AF = Acentric fragment
GT = Cells with 10 aberrations; counted as 1 aberration
NR = Not reported

*Significantly higher than the vehicle control group ($p<0.001$) by Wilcoxon's sum of ranks test

Note: Data were extracted from the study report pp. 17-24.

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DATA EVALUATION REPORT

BARDAC 22

Study Type: Mutagenicity: Mammalian Cells in Culture Cytogenetic Assay in Chinese Hamster Ovary (CHO) Cells

Prepared for:

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

Prepared by:

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

Principal Reviewer	Kristin Jacobson, MSPH	Date 11/9/93
Independent Reviewer	Nancy E. McCarroll, B.S.	Date 11/9/93
QA/QC Manager	Sharon Segal, Ph.D.	Date 11/6/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 293
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

EPA Reviewer: Robert Fricke, Ph.D. Signature: Robert L. Fricke
Review Section IV, Toxicology Branch II Date: 18 May 93
Health Effects Division (7509C)
EPA Section Head: Jess Rowland, M.S. Signature: Jess Rowland
Review Section IV, Toxicology Branch II Date: 11/19/93
Health Effects Division (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mammalian cells in culture cytogenetic assay in Chinese hamster ovary (CHO) cells (84-2)

TEST MATERIAL: BARDAC 22

PC CODE: 069149

DP BARCODES: D188679, D189620

CASWELL NO.: 331A

SUBMISSION NOS.: S436173

MRID Number: 412526-01 (Duplicate of MRID 407058-01, Doc. No. 8038)

SYNONYMS: Didecyldimethylammoniumchloride

SPONSOR: Lonza, Inc., Fair Lawn, NJ and Lonza AG, Basle Switzerland

TESTING FACILITY: Inveresk Research International, Musselburgh, Scotland

TITLE OF REPORT: P0151: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells in vitro

AUTHORS: M. Holmstrom, D.J. Leftwich, and I.A. Leddy

STUDY NUMBER: IRI Project No. 735717; IRI Report No. 4236

REPORT ISSUED: October 1986

CONCLUSIONS-EXECUTIVE SUMMARY:

> Negative for the induction of chromosome aberrations in Chinese hamster ovary (CHO) cells harvested 26 hours postexposure to 1-8 µg/mL -S9 or 2-8 µg/mL +S9. The test material was assayed to a cytotoxic level (≥16 µg/mL +/-S9).

CORE CLASSIFICATION: Acceptable. The study satisfies the Guideline requirements (84-2) for genetic effects, Category II, Structural Chromosome Aberrations, and is acceptable for regulatory purposes.

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A. MATERIALS:

1. Test Material: P0151

Description: Clear, colorless liquid
Identification number: Batch number E06130085
Purity: 50%
Receipt date: July 10, 1986
Stability: Not reported
Contaminants: None listed
Solvent used: Deionized distilled water (dH_2O)
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions were prepared immediately prior to use. Analytical determinations for achieved concentration or test material stability were not performed. The report did not indicate that solutions were adjusted to 100% purity.

2. Control Materials:

Negative: Untreated cells in culture medium (Ham's F12 medium with glutamine, supplemented with 15% fetal bovine serum (FBS) and antibiotics). The S9-activated treatments were conducted with serum-free medium.

Solvent/final concentration: dH_2O/1%

Positive:

Nonactivation: Methyl methanesulfonate (MMS) was prepared in dH_2O to yield a final concentration of 20 µg/mL.

Activation: Cyclophosphamide (CP) was prepared in dH_2O to yield a final concentration of 20 µg/mL, and 2-acetylaminofluorene (2-AAF) was prepared in dimethyl sulfoxide to yield a final concentration of 40 µg/mL.

3. Activation: S9 derived from male Fischer 344 (254-283 g)

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other:		____ other	

The S9 homogenate was prepared by the performing laboratory and assigned batch numbers FL1 017 and FL1 018. Prior to use, the total protein (25.9 mg/mL), concentration of cytochrome P450/P_1-450, and benzo(a)pyrene hydroxylase and N-demethylase activity were determined. In addition, the ability of the S9 fraction to convert five selected compounds to mutagens was assessed using Salmonella typhimurium strain TA1538. The contents of the S9 mix were as follows:

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Component	Final Concentration
Glucose-6-phosphate	25 mM
NADP	4 mM
KCl	223.7 mg/L
$MgSO_4$	147.9 mg/L
S9 homogenate	10%

4. Test Compound Concentrations Used:

   (a) Preliminary cytotoxicity assay: Nine half-log dilutions (0.8, 2.8, 8.4, 28, 84, 280, 839, 2796, and 8389 µg/mL +/- S9) were evaluated in single cultures per dose, per condition.

   (b) Cytogenetic assay: Duplicate cell cultures per dose, per condition were evaluated at the following dose levels:

   Nonactivated conditions:

   0.25, 0.5, 1, and 2 µg/mL (Aborted trial)
   1, 2, 4, and 8 µg/mL (Completed trial)

   S9-Activated conditions: 2, 4, 8, and 16 µg/mL

5. Test Cells: The Chinese hamster ovary cells ($CHO\text{-}K_1B_4$) used in this assay were obtained from Huntingdon Research Laboratories, Huntingdon, England. Prior to use, the CHO cells were maintained in complete Ham's F-12 medium.

   Properly maintained? Yes.
   Cell line or strain periodically checked for mycoplasma contamination? Not reported.
   Cell line or strain periodically check for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatment: Cells were exposed to the test compound, negative, solvent or positive control for: 24 hours (nonactivated), 6 hours (activated).

2. Preliminary Cytotoxicity Assay: Single cultures were exposed to the nine concentrations of the test material (0.8-8389 µg/mL +/- S9), or the solvent control. Under nonactivated conditions, cells were exposed to the test material for 24 hours. Cultures exposed to S9-activated test material doses were treated for 6 hours, washed, refed fresh culture medium and reincubated.

   Under either nonactivated or S9-activated conditions, cells were arrested in metaphase using colcemid (final concentration 0.1 µg/mL), 2 hours prior to harvest. Cultures were evaluated qualitatively for cytotoxicity and metaphases were harvested, fixed and stained with 5%

Giemsa. Mitotic indices (MIs) were determined from the number of metaphases observed in 1000 scored cells per culture. This scoring was performed first on the solvent control culture; cultures exposed to the test material were scored in order of descending concentration until the MIs of two consecutive doses were comparable to the solvent control MI.

3. Cytogenetic assay:

(a) Treatment: Duplicate cultures were prepared on the day prior to dosing, with a cell density of $1x10^5$ cells/culture. On the following day, cultures were exposed to the selected test material doses, negative, solvent or positive controls.

In the nonactivated assay, cells were treated for 24 hours. Cells exposed to the S9-activated doses of the test material were treated for 6 hours, washed twice, refed fresh culture medium and incubated for an additional 18 hours. Under both nonactivated and S9-activated conditions, 0.1 µg/mL colcemid was added to each culture 2 hours prior to cell harvest.

Metaphase cells were harvested, centrifuged, resuspended, fixed twice with methanol:glacial acetic acid (3:1), resuspended and spread onto slides (3 slides/culture). Slides were air-dried, stained with 5% Giemsa, rinsed, dried, cleared with xylene and coverslipped.

(b) Metaphase analysis: The slides were coded, and at least 100 cells from each of the duplicate treatment, negative, solvent or positive control cultures were scored for chromosomal aberrations. The number and type of structural and numerical aberrations and the frequency of cells with these aberrations were determined. Data were tabulated with and without gaps.

4. Statistical methods: The data were not evaluated for statistical significance.

5. Evaluation Criteria: The test material was considered positive if the number and frequency of aberrations was consistently above the reported historical background ranges (i.e., ≤0.12 lesions/cell or ≤4% aberrant cells excluding gaps and ≤9% aberrant cells including gaps), and the increases were dose-related and reproducible.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Under nonactivated conditions, compound precipitation was reported at concentrations ≥280 µg/mL and levels ≥2.8 µg/mL were severely cytotoxic. At the lowest dose (0.8 µg/mL), there were neither cytotoxic effects on the monolayers nor adverse effects on the MI. With S9-activation, the test material was less soluble (precipitation occurred at concentrations ≥28 µg/mL

and also less cytotoxic. At ≥28 µg/mL +S9, damage in 76-100% of cells was reported. Lower levels (≤8.4 µg/mL) were not cytotoxic.

Based on these findings, the concentrations selected for the cytogenetic assay were 0.25-2 µg/mL -S9 and 2-16 µg/mL +S9.

2. Cytogenetic Assay: Representative results from the nonactivated and S9-activated cytogenetic assay are presented in Table 1.

(a) Nonactivated conditions: The first nonactivated cytogenetic assay, conducted with 0.25-2 µg/mL P0151, was discarded due to the lack of cytotoxicity at the highest dose. Accordingly, a second assay was performed with nonactivated concentrations of 1-8 µg/mL. Cytotoxicity (reduced metaphases available for analysis) was noted in the 8-µg/mL culture. There were, however, no appreciable increases in the number or frequency of structural or numerical chromosome aberrations.

(b) S9-activated conditions: The highest S9-activated dose, 16 µg/mL, was severely cytotoxic; lower levels (2-8 µg/mL +S9) were not. The slight increase in the frequency of aberrant cells seen at 8 µg/mL was confined to this dose and was within the reporting laboratory's historical range (0.00-0.12 structural aberrations per cell). The data are, therefore, not indicative of a clastogenic response. There was also no consistent evidence of an adverse effect on chromosome number.

The frequency of structural aberrations in cultures treated with 40 µg/mL 2-AAF did not exceed the historical background rate for the reporting laboratory. The study authors stated that the lack of a response may have been due to the use of too low a concentration of 2-AAF. We conclude that the marginal activity observed with this compound, which is infrequently used as a positive control for this genetic endpoint, probably did not affect the outcome of the study. The sensitivity of the test system to detect a clastogenic response was adequately demonstrated by the increased yield of cells with aberrations following exposure to the positive controls conventionally applied in the CHO cytogenetic assay (20 µg/mL MMS -S9 and 20 µg/mL CP +S9).

Based on the overall results, the study authors concluded that P0151 was not clastogenic in this cytogenetic assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the results was correct. The test material, P0151, was assayed to cytotoxic levels (≥16 µg/mL +/-S9), but did not induce a clastogenic response in cultured CHO cells. In addition, the sensitivity of the test system to detect a clastogenic effect was adequately demonstrated by the marked increase in aberrations induced by the nonactivated (MMS) and S9-activated (CP) positive controls. We conclude, therefore, that P0151 is not a mutagen in this in vitro cytogenetic assay.

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MAMMALIAN CELLS IN CULTURE CYTOGENETICS

Table 1

							(%)	Statistically Significant Aberrations (No/Type)[b]
			[illegible]	3	0.025	2.5	4.0	1TB, 1TF, 3SF, 1AE, 7PP
			[illegible]	3	0.060	1.5	6.0	1TB, 1SF, 1EXCH, 4AE, 8PP
Deionized water	11	-	200	5	0.025	1.0	6.5	3TB, 1TF, 1SF, 13PP
		+	200	1	0.075	0.5	6.5	1SF, 6AE, 7PP
Positive Control								
Methylmethane sulfonate	20 µg/mL	-	200	41	0.205	16.5	7.0	14TB, 3TF, 3SF, 21 EXCH, 4AE, 1ER, 9PP
Cyclophosphamide	20 µg/mL	+	200	37	0.185	13.0	9.5	8TB, 11TF, 5SF, 12EXCH, 1R, 9AE, 10PP
2-Acetylamino-fluorene	40 µg/mL	+	200	12	0.060	4.5	6.5	3TB, 4TF, 4EXCH, 1R, 2AE, 3ER, 8PP
Test Material								
F9151	4 µg/mL[c]	-	200	8	0.040	3.0	5.0	1TB, 1TF, 5SF, 1R, 1ER, 9PP
	8 µg/mL[d]	-	52	1	0.019	1.9	7.7	1TF, 4PP
	8 µg/mL[c,e]	+	200	10	0.050	3.5	5.0	1TB, 3TF, 4SF, 2EXCH, 4ER, 6PP

[a]Gaps excluded

[b]Abbreviations used:

Structural aberrations. TB = Chromatid break; TF = Chromatid fragment; SB = Chromosome break; SF = Chromosome fragment; EXCH = Exchange; R = Ring

Numerical aberrations: AE = Aneuploidy; ER = Endoreduplication, PP = Polyploidy

[c]Results for lower doses (1 and 2 µg/mL -S9; 2 and 4 µg/mL +S9, did not suggest a clastogenic effect.

[d]The number of metaphases available for analysis was reduced due to cytotoxicity at this dose.

[e]The highest level (16 µg/mL +S9) was severely cytotoxic.

Note: Data were extracted from the study report: see CBI pp. 29-34.

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[redacted]

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement, signed and dated November 24, 1986, stated that the report was audited and the methods involved in this type study are inspected on a pre-determined schedule.)

CORE CLASSIFICATION: Acceptable. The study satisfies the Guideline requirements (84-2) for genetic effects, Category II, Structural Chromosome Aberrations, and is acceptable for regulatory purposes.

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010689

FINAL

DATA EVALUATION REPORT

BARDAC 22

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT)

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
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Contract Number: 68D10075
Work Assignment Number: 2-101
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010689

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

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Health Effects Division (7509C)
Signature: Jess Rowland
Date: 11/19/93

DATA EVALUATION REPORT

STUDY TYPE: Gene mutation in cultured Chinese hamster ovary cells (CHO/HGPRT) (84-2)

TEST MATERIAL: BARDAC 22

PC CODE: 069149

DP BARCODES: D188679, D189620

SUBMISSION NO.: S436173

CASWELL NO.: 331A

MRID Number: 408952-02

SYNONYM(S): Didecyldimethylammoniumchloride (DDAC)

SPONSOR: Lonza, Inc., Fair Lawn, NJ

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD

TITLE OF REPORT: Mutagenicity Test on Didecyldimethylammonium chloride (DDAC) in the CHO/HGPRT Forward Mutation Assay

AUTHOR: R.R. Young

STUDY NUMBER: HLA Study No. 10141-0-435

REPORT ISSUED: September 9, 1988

CONCLUSIONS--EXECUTIVE SUMMARY:

> Negative for the induction of forward gene mutations in Chinese hamster ovary (CHO) cells at the HGPRT locus in two independent assays conducted with levels ranging from 1-10 µg/mL -S9 and 1-26 µg/mL +S9. Severe cytotoxicity was demonstrated at doses ≥10 µg/mL -S9 and ≥25 µg/mL +S9.

CORE CLASSIFICATION: Acceptable. The study satisfies the Guideline requirement (84-2) for genetic effects, Category I, Gene Mutations, and is acceptable for regulatory purposes.

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MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS

1. Test Material: BARDAC 22

Description: Clear, slightly viscous yellow liquid
Identification number: Lot number B-1889
Purity: 80%
Receipt date: October 21, 1987
Stability: Stable in the solvent for 24 hours at 5°C
Contaminants: None listed
Solvent used: Deionized water (dH_2O)
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions were prepared on the day of testing. Actual test material concentrations were determined on the primary stock solutions for each mutation assay; in addition, the 24-hour stability of the test material in the solvent was analytically verified. The report did not indicate that solutions were adjusted to 100% purity.

2. Culture Medium: Ham's Nutrient Mixture F12 containing 10% fetal bovine serum (FBS), L-glutamine and antibiotics. For the S9-activated assays, the FBS concentration was reduced to 5%.

3. Control Materials:

Negative: Culture medium (cytotoxicity assay only)

Solvent: dH_2O, at a final concentration of 10%

Positive: Nonactivation (concentrations, solvent): 5-Bromo-2'-deoxyuridine (BrdU) was prepared in an unspecified solvent to yield a final concentration of 50 µg/mL.

Activation (concentrations, solvent): 3-Methylcholanthrene (3-MC) was prepared in an unspecified solvent to yield a final concentration of 5 µg/mL.

4. Activation: S9 derived from Sprague-Dawley (sex not specified)

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 homogenate was purchased commercially (supplier not specified). Prior to use, CHO/HGPRT assays were conducted with two reference mutagens (benzo(a)pyrene and 3-MC) and various concentrations of S9 in order to select the optimum S9 concentration. The S9 mix was prepared as follows:

DOE

Component	Final Concentration
Phosphate buffer	2.0 mM
NADP	1.0 mM
Glucose-6-phosphate	5.0 mM
$CaCl_2$	2.0 mM
KCl	6.6 mM
$MgCl_2$	2.0 mM
S9 homogenate	20.0 µL/mL (2%)

5. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
_x_ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Yes.
Periodically "cleansed" against high spontaneous background? Yes.

6. Locus Examined:

_____ thymidine kinase (TK).
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

_x_ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) _4 µg/mL_ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

7. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Ten doses (5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 µg/mL) were initially evaluated in the presence and absence of S9 activation, using triplicate cultures. Due to severe cytotoxicity at all nonactivated levels, a second preliminary cytotoxicity assay was conducted with a lower range of nonactivated concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, and 8.0 µg/mL). An additional nonactivated preliminary study was conducted with dose levels of 0.05, 0.1, 0.2, 1.0, 2.0, 5.0, 10, 20, and 50 µg/mL using a high

density cell monolayer (1.0×10^6 cells). The objective of this additional preliminary study was to determine if the severity of test material cytotoxicity would be reproduced under the actual conditions of the mutation assay. This assessment entailed a qualitative evaluation of the monolayer confluence.

(b) Mutation assay: Two independent assays were conducted with single cultures in the presence or absence of S9 activation, as follows:

(1) Nonactivated conditions:

Initial trial: 1, 2, 3, 4, 5, 6, 7, 8, 10, and 13 µg/mL. Cells exposed to levels between 3-8 µg/mL were plated for selection.

Repeat trial: Doses were equivalent to those used in the initial trial. Cultures exposed to concentrations between 3-10 µg/mL were plated for selection.

(2) S9-activated conditions:

Initial trial: 1, 5, 10, 13, 15, 18, 20, 25, 30, and 40 µg/mL. Cultures exposed to the levels between 5-25 µg/mL were plated for selection.

Repeat trial: 1, 5, 10, 18, 20, 22, 26, and 30 µg/mL. Cultures exposed to 1-22 µg/mL were plated for selection.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells were exposed to the test compound, solvent or positive control for: 4 hours (nonactivated or S9-activated conditions)

(b) After washing, cells were cultured for 7 days (expression period) before cell selection.

(c) After expression, 2×10^5 cells/dish (12 dishes/culture) were cultured for 7-10 days in selection medium to determine numbers of mutants; 200 cells/dish (3 dishes/culture) were cultured for 7-10 days in nonselection medium to determine cloning efficiency (CE)

2. Statistical Methods: Mutation frequency (MF) data were analyzed for statistical significance ($p \leq 0.05$ and 0.01) using a two-tailed binomial approximation of a Poisson distribution, using the tables of Kastenbaum and Bowman (1970).[1]

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the following conditions were met: (1) the CE in the vehicle controls was between 50-115%; (2) the MF in the vehicle controls was $\leq 15 \times 10^{-6}$; (3) the MF of the positive control was significantly ($p \leq 0.01$) higher than the solvent control; (4) the test material was tested either to the maximum concentration, to a concentration approximately twice that of the test material's solubility limit in the culture medium, to a concentration that reduced relative survival (RS) to 85-90%, or to 75% of a highly cytotoxic concentration; (5) the MF at each dose level was based on the counts from at least eight of twelve mutant selection dishes and from 2 of 3 cloning efficiency dishes; and (6) at least three dose levels were evaluated for induction of mutations.

(b) Positive response: The test material was considered positive if a significant ($p \leq 0.05$), dose-related increase in the MF above 15×10^{-6} was observed, or if there was a >2-fold increase at a single dose level near the cytotoxicity limit. Smaller increases (i.e., 2-fold) must have been reproduced in a confirmatory assay.

C. REPORTED RESULTS:

1. Test Material Solubility/Solution Preparation: The test material was reported to be soluble in dH_2O up to 50 mg/mL. At concentrations ≥200 µg/mL, a white precipitate was reported in the culture medium. No pH alterations in the culture medium were caused by the test material. The study author stated that all primary stock solutions were prepared in pipets and tubes. For the mutation assays, test solutions were prepared with glass pipets and volumetric flasks.

2. Preliminary Cytotoxicity Test: In the first cytotoxicity assay, all nonactivated concentrations (5-5000 µg/mL) were severely cytotoxic; data from this nonactivated assay were not reported. In the second cytotoxicity assay, concentrations ≤2 µg/mL were nontoxic (RS ≥93.7%); however, ≤1.9% of the cells survived treatment with ≥4 µg/mL. Results from the third nonactivated cytotoxicity assay demonstrated a slight cell density-dependent reduction in cytotoxicity, as determined from a qualitative evaluation of monolayer confluence. No effects on

[1]Kastenbaum, M.A. and Bowman, K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat Res 9: 527-549.

confluence were seen in monolayers exposed to 0.5-2 µg/mL; a 50% reduction in confluence was noted at 5 µg/mL, and no cells were visible at concentrations ≥10 µg/mL.

The S9-activated test material was less cytotoxic than the nonactivated test material. A single S9-activated cytotoxicity test was conducted, in which the two lowest doses (5 and 10 µg/mL) were noncytotoxic (RS ≥100%) and all higher levels (≥20 µg/mL) were lethal (0% RS).

Based on these findings, doses representing a cytotoxicity range of approximately 0-90% (1-13 µg/mL -S9 and 1-40 µg/mL +S9) were selected for the initial mutation assay.

3. Mutation Assays:

(a) Nonactivated conditions: Representative results from the two nonactivated assays are presented in Table 1. Severe cytotoxicity (i.e., ≤1% survival) occurred in the 10- and 13-µg/mL cultures; these cultures were, therefore, not plated for mutant selection. For the remaining concentrations, RS was dose related and ranged from 10.7% at 8.0 µg/mL to ≥100% at ≤3 µg/mL. There was, however, no evidence of a mutagenic effect. In the repeat nonactivated trial, cytotoxicity was less severe in the 10-µg/mL culture (5.7% RS, in contrast to 0.4% RS in the initial trial); accordingly, cells treated with this dose were also plated for selection. In agreement with the initial assay, there were no appreciable increases ($\geq 15 \times 10^{-6}$) in the MF at any dose.

(b) S9-activated conditions: Table 2 presents representative data from the two S9-activated assays. In both the initial and repeat trials, severe cytotoxicity (RS ≤3%) was reported in cultures treated with ≥25 µg/mL +S9. There were, however, no appreciable increases in the MF of any treated cultures.

In contrast, the positive controls (nonactivated 50 µg/mL BrdU and S9-activated 5 µg/mL 3-MC) induced significant ($p \leq 0.01$) increases in the MFs in both trials.

4. Analytical Determinations: Using spectrophotometry, duplicate samples of the test material primary stock solutions for each of the mutation assays were analyzed for actual concentration. All samples were within 4% of the respective target concentrations. In addition, the 24-hour stability of the test material (at 5°C) was verified for solutions containing 1, 10, or 100 µg/mL test material.

Based on the overall findings, the study author concluded that DDAC was not mutagenic in the CHO/HGPRT forward gene mutation assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that [redacted] DACDAC 22 was tested over a range of concentrations that included severely cytotoxic doses (≥10 µg/mL -S9 and ≥25 µg/mL +S9) but failed to induce a mutagenic response. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the nonactivated (50 µg/mL BrdU) and S9-activated (5 µg/mL 3-MC) positive controls. We conclude, therefore, that [redacted] is not a mutagen in this *in vitro* mammalian cell gene mutation assay. DACDAC 22

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated September 9, 1988.)

CORE CLASSIFICATION: Acceptable. The study satisfies the Guideline requirement (84-2) for genetic effects, Category I, Gene Mutations, and is acceptable for regulatory purposes.

Table 1. Representative Results of the Nonactivated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with BARDAC 22

Substance	Dose	Relative Percent Cloning Efficiency (after treatment)[a]	Number of Mutant Colonies	Absolute Percent Cloning Efficiency (at selection)[a]	Mutation Frequency x10^{-6}[b]
Solvent Control[c]					
Deionized water	10%	100.0 (95.0)[d] 100.0 (91.0)[e]	14 5	0.88 1.07	6.6 1.9
Positive Control					
5-Bromo-2'-deoxy-uridine	50 µg/mL	77.9[d] 78.2[e]	220 327	0.72 1.00	133.1[f] 136.2[f]
Test Material					
BBAC	7 µg/mL[g] 8 µg/mL[g]	42.3[d] 18.7	11 5	0.86 0.84	5.3 2.5
	7 µg/mL[g] 8 µg/mL 10 µg/mL[g]	22.5[e] 9.3 5.7	9 1 8	0.97 1.02 1.00	3.9 0.4 3.3

[a]Based on the results of single cultures per treatment or positive control group and duplicate cultures in the solvent control group. Values in () are absolute survival; calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{(\text{No. of Dishes, 12})(\text{No. of Cells Plated, } 1\text{x}10^5)(\text{Cloning Efficiency})}$; calculated by our reviewers.

[c]Solvent control values represent the average value from duplicate cultures; calculated by our reviewers.
[d]Results from the initial trial
[e]Results from the repeat trial
[f]Findings for lower doses (1, 2, 3, 4, 5, and 6 µg/mL -- initial and repeat trials) did not suggest a mutagenic response.
[g]Higher doses (10 and 12 µg/mL -- initial trial; 12 µg/mL -- repeat trial) were severely cytotoxic and were, therefore, not plated for selection.

*Significant ($p \leq 0.01$) by 2-tailed binomial test (i.e., Kastenbaum-Bowman tables) and higher than the acceptable background range of <13x10^{-6}

Note: Data were extracted from the study report, pp. 30-31.

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Table 1

MANHATTAN CHEMICAL CULTURES CHINESE HAMSTER OVARY MUTATION

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Table 2. Representative Results of the S9-activated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with BARBAC 22

Substance	Dose	Relative Percent Cloning Efficiency (after treatment)[a]	Number of Mutant Colonies	Absolute Percent Cloning Efficiency (at selection)[a]	Mutation Frequency $x10^{-6}$[b]
Solvent Control[c]					
Deionized water	10%	100.0 (94.8)[d]	4	1.02	1.6
		100.0 (93.2)[e]	4	1.16	1.4
Positive Control					
3-Methylcholanthrene	5 µg/mL	93.3[d]	232[f]	0.95	122.1[a,f]
		93.1[e]	348	0.99	146.2[a]
Test Material					
BCMC	20 µg/mL[g]	30.4[d]	4	0.99	1.7
	25 µg/mL[h]	2.8	2	0.92	2.9
	28 µg/mL[g]	33.4[e]	30	1.06	11.8
	22 µg/mL[h]	19.3	1	1.03	8.4

[a]Based on the results of single cultures per treatment or positive control group and duplicate cultures in the solvent control group. Values in () are absolute survival; calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{(\text{No. of Dishes, 12})(\text{No. of Cells Plated, } 2\text{x}10^5)(\text{Cloning Efficiency})}$; calculated by our reviewers.

[c]Solvent control values represent the average value from duplicate cultures; calculated by our reviewers.
[d]Results from the initial trial
[e]Results from the repeat trial
[f]Based on counts from 10 plates; the other two plates were lost due to contamination.
[g]Findings for lower doses (1, 5, 10, 13, 15, and 18 µg/mL -- initial trial; 1, 5, 10, 15, and 18 µg/mL -- repeat trial) did not suggest a mutagenic response.
[h]Higher doses (36 and 45 µg/mL -- initial trial; 26 and 30 µg/mL -- repeat trial) were severely cytotoxic and were, therefore, not plated for selection.

[a]Significant ($p \leq 0.01$) by 2-tailed binomial test (i.e., Kastenbaum-Bowman tables) and higher than the acceptable background range of $<15x10^{-6}$

Note: Data were extracted from the study report, pp. 32-33.

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Table 2

MAMMALIAN CELLS IN CULTURE GENE MUTATION

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FINAL

DATA EVALUATION REPORT

DIDECYLDIMETHYLAMMONIUMCHLORIDE (DDAC)

Study Type: Mutagenicity: Unscheduled DNA Synthesis (UDS) Assay in Primary Rat Hepatocytes (84-4)

Prepared for:

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

Prepared by:

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

Principal Reviewer — Dean Walton, Ph.D. — Date 11/1/93

Independent Reviewer — Nancy E. McCarroll, B.S. — Date 11/1/93

QA/QC Manager — Sharon Segal, Ph.D. — Date 11/11/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 296
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY UDS

MUTAGENICITY STUDIES

EPA Reviewer: Robert Fricke, Ph.D. Signature: Robert J. Fricke
Review Section IV, Date: 18 Nov 93
Toxicology Branch IV/HED H7509C
EPA Section Head: Jess Rowland, M.S. Signature: Jess Rowland
Review Section IV, Date: 11/19/93
Toxicology Branch IV/HED H7509C

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes.

CHEMICAL: BARDAC 22

DP BARCODES: DP188679, DP189620 Submission No.: S436173

Caswell No.: 331A

MRID Number: 408952-01

SYNONYM/CAS No.: Didecyldimethylammoniumchloride (DDAC)

SPONSOR: Lonza, Inc., Fair Lawn, NJ

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD

TITLE OF REPORT: Mutagenicity Test on Didecyldimethylammoniumchloride in the Rat Primary Hepatocyte Assay

AUTHOR: M.A. Cifone

STUDY NUMBER: 10141-0-447

REPORT ISSUED: September 12, 1988

CONCLUSIONS-EXECUTIVE SUMMARY: Negative for inducing unscheduled DNA synthesis (UDS) in primary rat hepatocytes treated with the test material at doses up to 2.00 µg/mL. Higher concentrations (≥4.00 µg/mL) were severely cytotoxic.

CORE CLASSIFICATION: Acceptable. This study satisfies the guideline requirements for an in vitro (UDS) mutagenicity study (84-4) and is acceptable for regulatory purposes.

D/3

A MATERIALS:

1. Test Material: DDAC

Description: Viscous, clear yellow liquid
Identification number: Batch number B1889
Purity: 80%
Receipt date: October 21, 1987
Stability: Stable for at least 24 hours in an aqueous solution at 5°C (see Section C, Reported Results).
Contaminants: Not reported
Solvent used: Deionized water (dH_2O)
Other provided information: Prior to each trial, stock solutions of the test material were prepared using volumetric flasks and pipets. Prepared solutions containing 1-100 µg/mL were analyzed for stability and all dosing solutions used in both trials were analyzed to verify target concentrations.

2. Indicator Cells: Rat hepatocytes harvested from 150-300 g adult male rats (Strain: Fischer 344) that were purchased from Charles River Breeding Laboratories, Inc. Single rats were used for each trial.

3. Control Substances:

(a) The solvent control was sterile dH_2O at a final concentration in the media of 10%.

(b) The positive control was 4.48×10^{-7} M (0.10 µg/mL) acetylaminofluorene (2-AAF)

4. Medium: William's Medium E (WME+) contained 5% fetal bovine serum, 2 mM L-glutamine, 2.4 µM dexamethasone, and antibiotics. WME: as above without dexamethasone.

5. Test Compound Concentrations Used:

Cytotoxicity Assay: An independent cytotoxicity assay was performed using log-fold dilutions of the test material ranging from 100 µg/mL to 0.100 µg/mL.

UDS Assays:

(a) Initial assay: Eleven doses ranging from 0.05 µg/mL to 10 µg/mL were evaluated. Cells exposed to 0.05, 0.100, 0.250, 0.750, 1.00, and 2.00 µg/mL were scored.

(b) Repeat Assay: In the repeat assay, doses comparable to those used in the initial trial were evaluated and cells exposed to 0.100, 0.250, 0.500, 0.750, 1.00, and 2.00 µg/mL were scored.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion technique: The livers were perfused in situ with Hank's balanced salts containing 0.5 mM EGTA and HEPES buffer solution (pH 7.2) for 4 minutes and with 50-100 U/mL collagenase for 10 minutes. Each liver was excised, removed to a culture dish containing WME and collagenase and mechanically dispersed to release the hepatocytes.

(b) Hepatocyte harvest/culture preparation: Recovered cells were centrifuged, resuspended in WME+, counted, and ~0.5×10^6 cells/3 mL were dispensed into culture dishes containing plastic coverslips. The cultures were placed in a humidified, 37°C, 5% CO_2 incubator for a 1.5-2.0 hour attachment period. Unattached cells were removed; viable cells were refed and established as monolayer cultures.

2. UDS Assay:

(a) Treatment: Five replicate monolayer cultures were exposed to the selective doses of the test material, negative or positive controls (2-AAF) for 18-19 hours in WME containing 5 µCi/mL [^{3}H]-thymidine (specific activity of 20 Ci/mmol) and 1% fetal bovine serum. Treated monolayers were washed twice with WME: two of the five replicates for each treatment group were used to determine cytotoxicity. These cultures were refed, re-incubated, and at ~20 hours posttreatment, monitored for cytotoxicity by trypan blue exclusion.

(b) UDS slide preparation: The remaining cultures were washed with media containing 1 mM thymidine. Treated hepatocytes, attached to coverslips were swollen with 1% sodium citrate and fixed in ethanol:acetic acid (3:1). The coverslips were rinsed and mounted onto slides; each slide was dipped in NTB2 photographic emulsion (Kodak) and dried.

(c) Preparation of autoradiographs/grain development: Slides were exposed for 7-10 days at 4°C in light-proof boxes containing desiccant, developed with Kodak D-19 fixed and stained with Williams' modified hematoxylin-eosin. Slides were coded prior to analysis.

(d) Grain counting: The nuclear and cytoplasmic grains of 150 cells (50/coverslip) per treatment were counted. The net nuclear grain counts were quantitated by subtracting the highest cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus from the nuclear grain count. Cells in S-phase were not scored.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if: (1) hepatocytes recovered from the perfusion step and monolayer cultures used for the assay reached a ≥70% viability level; (2) the negative control had a net nuclear grain count of -5.0 to 1.0, no more than 10% of the cell contained ≥6 grains and no more than 1% of the cells contained ≥20 grains; (3) the positive control demonstrated the sensitivity of the test system to detect UDS; (4) data were obtained from at least two replicate cultures/dose; and (5) the highest dose was cytotoxic, limited by solubility, or reached the maximum recommended dose for this assay (5000 µg/mL).

(b) Positive response: The test material was considered positive if: (1) the increase in the mean nuclear grain count was ≥6 grains/nucleus over the negative control value; (2) the percentage of nuclei with ≥6 grains exceeded 10% of the negative control population; or (3) the percentage of nuclei with ≥20 grains was ≥2% of the analyzed population.

4. Statistical Methods: The data were not analyzed for statistical significance.

C. REPORTED RESULTS:

1. Cytotoxicity Assays: The study author stated that 10 mg/mL DDAC was soluble in dH_2O, forming a clear, colorless solution which foamed when shaken vigorously. Doses ranging from 0.10 µg/mL to 100 µg/mL of the test material were examined in the cytotoxicity assay. No data were provided; however, the report stated that the highest dose for the UDS assay was selected as the next level above the dose where cell morphology was nearly normal and viability was ~50% of the solvent control.

2. Initial UDS Assay: Eleven doses ranging from 0.05 µg/mL to 10.0 µg/mL were assayed in the initial trial. The study author reported that doses ≥4.00 µg/mL were "excessively toxic." Accordingly, six doses, ranging from 0.050 µg/mL to 2.00 µg/mL of DDAC were scored. The data presented in Table 1 indicate that hepatocyte viability was dose dependent and ranged from 74.6% at the highest scored level (2.0 µg/mL) to >99% at 0.75 µg/mL. The selected doses of DDAC (0.05, 0.10, 0.25, 0.75, 1.00, or 2.00 µg/mL) did not induce a genotoxic effect. Hepatocytes exposed to 0.50 µg/mL were not scored because of poor cell attachment. Although, slight increases in net nuclear grains and the percentage of cells with ≥6 net nuclear grains were noted at the majority of doses, the effect was not concentration dependent and was not considered to be indicative of a positive response. By contrast to the lack of genotoxic effects with DDAC, marked increases in the net nuclear grain counts and the percentage of cells in repair were observed in hepatocytes treated with the positive control (0.100 µg/mL 2-AAF).

TABLE 1. Representative Results of the Initial Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assay with Didecyldimethylammoniumchloride

Treatment	Dose	Number of Cells Scored/ Group	Net Nuclear Grains[a]	Percent of Cells in Repair (≥6 Net Nuclear Grains)	Percent Survival at 20 hours
Solvent Control					
Deionized water	10%	150	-0.19	0.0	100.0
Positive Control					
2-Acetylaminofluorene	0.10 µg/mL	150	14.62	94.0	97.6
Test Material					
Didecyldimethyl-chloride	0.75 µg/mL[b]	150	0.69	2.7	99.7
	1.00 µg/mL	150	0.85	3.0	85.1
	2.00 µg/mL	150	0.33	4.7	74.6

[a]Mean and standard deviations of net nuclear grain counts for 150 cells; 50/slide from 3 slides for the treatment and negative control groups; 75 cells/2 slides from the positive control

[b]Lower doses (0.50, 0.10, or 0.25 µg/mL test material) did not suggest a genotoxic effect; grain counts from the 0.50-µg/mL dose group were not scored because of poor cell attachment.

Note: Data were extracted from the study report pp. 23 and 27.

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UDS

3. Repeat UDS Assay: For the repeat trial, equivalent DDAC levels were assayed and cells treated with 0.10, 0.25, 0.50, 0.75, 1.00, or 2.00 µg/mL were scored. As the data presented in Table 2 show, the findings from the repeat trial confirmed the earlier results indicating that DDAC was assayed to cytotoxic doses but did not induce UDS. The positive control produced a marked increase in the number of net nuclear grains and cells in repair.

4. Analytical Determinants: Results of the stability determination of stock solutions containing 1, 10, or 100 µg/mL indicated that the test material was stable in dH_2O for 24 hours at 5°C. Similarly, the analysis of dosing solutions prepared for both UDS assays revealed that with the exception of the lowest concentration (1.0 µg/mL) used in both trials, all solutions were accurately prepared and were within ~6% of the target level.

Based on the overall results, the study author concluded that DDAC was not genotoxic in this test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We agree with the study author's conclusions that DDAC did not induce genotoxic effects at the concentrations tested in this rat hepatocyte DNA repair assay. We further assess that the material was assayed over an appropriate range that included cytotoxic levels; therefore, the assay provided valid evidence of a negative response in this test system. In addition, the sensitivity of the test system to detect UDS was adequately demonstrated by the results obtained with the positive control (0.100 µg/mL 2-AAF).

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated September 12, 1988.)

CORE CLASSIFICATION: Acceptable. The study satisfies the guideline requirements for an in vitro (UDS) mutagenicity study (84-4) and is acceptable for regulatory purposes.

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TABLE 2. Representative results of the Repeat Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assay with Didecyldimethylammonium chloride

Treatment	Dose	Number of Cells Scored/ Group	Net Nuclear Grains[a]	Percent of Cells in Repair (≥6 Net Nuclear Grains)	Percent Survival at 20 hours
Solvent Control					100.0
Deionized water	10%	300	0.32	6.3	
Positive Control					101.0
2-Acetylaminofluorene	0.10 µg/mL	150	18.12	96.0	
Test Material					96.9
Didecyldimethyl-ammoniumchloride	0.75 µg/mL[b]	150	-0.27	5.3	102.4
	1.00 µg/mL	150	0.15	0.7	85.6
	2.00 µg/mL	150	-0.39	2.0	

[a]Mean and standard deviations of net nuclear grain counts for 300 cells (solvent control group) or 150 cells (treatment groups); 100 or 50 cells from each of the three slides per group were analyzed from the solvent and treatment groups, respectively; 75 cells/2 slides were scored from the positive control group

[b]Lower doses (0.10, 0.25, or 0.50 µg/ml) did not suggest a genotoxic effect.

Note: Data were extracted from the study report pp. 24 and 28.

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010688

FINAL

DATA EVALUATION REPORT

BARDAC 22

Study Type: Metabolism

Prepared for:

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

Prepared by:

Clement International Corporation
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Fairfax, VA 22031-1207

Principal Reviewer: Karen N. Gan Date 11/11/93
Karen Gan, M.S.

Independent Reviewer: Sanju Diwan Date 11/11/93
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QA/QC Manager: Sharon A. Segal Date 11/12/93
Sharon A. Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 300, 301
Project Officer: Caroline Gordon

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EPA Reviewer: Robert Fricke, Ph.D.
Review Section IV, Toxicology Branch II,
Health Effects Division

Signature: Robert L Fricke
Date: 19 Nov 92

EPA Section Head: Jess Rowland, M.S.
Review Section IV, Toxicology Branch II

Signature: Jess Rowland
Date: 11/19/92

DATA EVALUATION REPORT

010639

STUDY TYPE: Metabolism in Rats (Guideline Series 85-1)

EPA IDENTIFICATION NUMBERS:

DP Barcodes: D188679, D189620

Submission No.: S436173

P.C. Code: 069149

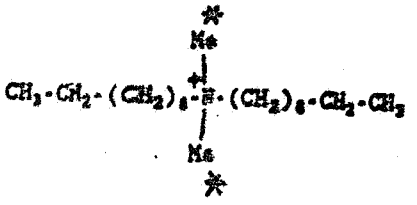
Caswell No.: 331A

MRID Numbers: 416171-01, 413851-01

TEST MATERIAL: BARDAC 22

SYNONYM: Didecyldimethylammoniumchloride; DDAC

CHEMICAL STRUCTURE:



* denotes the position of the ^{14}C label

SPONSOR: Lonza, Inc., Fair Lawn, NJ

PERFORMING LABORATORY: Biological Test Center, Irvine, CA

AUTHORS: Sami Selim (Report 1); Paul Lin and Sami Selim (Report 2)

REPORTS: 1) Absorption, Distribution, Metabolism and Excretion Studies of Didecyldimethylammoniumchloride (DDAC) in the Rat. BTC Study No. P01421. MRID# 416171-01.

2) Addendum to report entitled "Absorption, Distribution, Metabolism and Excretion Studies of Didecyldimethylammoniumchloride (BARDAC 22) in the Rat." Addendum to BTC Study No. P01421. MRID# 413851-01.

STUDY COMPLETION DATES: December 1, 1989 (Report 1); December 18, 1989 (Report 2)

CONCLUSIONS: The absorption, distribution, metabolism, and excretion of [^{14}C]-BARDAC 22 were studied in groups of rats (5/sex/group) administered a single oral gavage dose of 5 or 50 mg/kg [^{14}C]-BARDAC 22 or a 14-day diet containing 5 mg/kg unlabeled BARDAC 22 followed by a single oral gavage dose of 5 mg/kg [^{14}C]-BARDAC 22 on day 15. A preliminary study was conducted to determine recovery of expired volatile ^{14}C-residues in 2 male and 2 female rats after administration of a single oral dose of ≈4.7 mg/kg [^{14}C]-BARDAC 22.

The study demonstrated that ^{14}C-BARDAC 22 was metabolized and excreted following oral administration in rats. Total recoveries of the radioactivity were high for most groups (90.82-100.94% of administered dose). Most of the radioactivity in all groups was recovered in the feces (89.11-99.46% of administered dose), however, the amounts represented by biliary excretion and unabsorbed test material are not known because intravenous dosing was not conducted. Bioaccumulation of BARDAC 22 appeared to be low as indicated by <1% recovery in tissues at 7 days postdosing. Metabolism data suggest that BARDAC 22 is oxidized at the two decyl side chains to form hydroxy and hydroxyketo derivatives. Mass spectrometric analyses indicated that the 2 methyl substituents of BARDAC 22 remained unmodified. Furthermore, the hydroxy and/or keto functions on the decyl side chains are probably located at or near the terminal end. Greater metabolism (as indicated by decreased amount of parent compound in feces) occurred in females than males and in the low-dose groups than high-dose group.

Therefore, no sex- or dose-related differences were found in the distribution and elimination of BARDAC 22, however, slight sex- and dose-related differences were seen in the metabolism of BARDAC 22.

CORE CLASSIFICATION: Supplementary. This study may be upgraded if an intravenous study is conducted (to determine the extent of oral absorption of BARDAC 22) and is judged to be acceptable. This does not satisfy guideline requirement (85-1) for a metabolism study and is not acceptable for regulatory purposes.

A. MATERIALS

1. Test Substance

The nonradiolabeled test material (lot number 7807-E) in 40% aqueous solution was prepared by Rutgers University, New Brunswick, NJ, and had a purity of 99.4%. In the repeated-dose study, nonradiolabeled Bardac 2280® technical herbicide was added to the diet of rats for 14 days prior to a radiolabeled dose of BARDAC 22. The diets contained 34 ppm BARDAC 22.

Radiolabeled BARDAC 22 (lot number 7499-E) had a radiopurity of 99.0%. The test material was labeled with ^{14}C at a N-methyl group. Calculations made in the study were based on a reported specific activity of 9.01 mCi/mmol. It was later determined that the actual specific activity was 9.40 mCi/mmol; however, investigators considered the differences in values to be small and that the error equally affected both dosed and recovered data.

2. Test Animals

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Six-week-old Sprague-Dawley rats (5/sex/group) were obtained from Charles River Laboratories, Portage, MI. Mean body weights at the initiation of the study were 310.8-322.8 g for males and 212.8-214.8 g for females. A single oral gavage dose of 5 mg/kg (low-dose group) or 50 mg/kg (high-dose group) labeled BARDAC 22 was administered to rats. Another group received 34 ppm unlabeled BARDAC 22 in the diet daily for 14 days followed by a single oral gavage dose of 5 mg/kg radiolabeled BARDAC 22 (repeated-dose group). An additional group of 10 male rats (236 to 262 g) were used to collect a sufficient amount of ^{14}C fecal residue for metabolite analysis. In the preliminary $^{14}CO_2$ study, rats (2/sex) received a single oral gavage dose of 4.7-4.8 mg/kg. Mean body weights were 193.5 and 137.0 g for males and females, respectively. The rationale for the chosen doses was not reported.

B. METHODS

1. Acclimation

Rats were acclimated in individual cages for two weeks prior to use. Animals were provided Purina® Rat Chow (#5002) and water ad libitum throughout the study except 18 hours prior to dosing until 4 hours postdosing. Animals (2/sex) for the expired $^{14}CO_2$ study were kept in individual glass metabolism cages (Roth Cage). Animals were then kept in individual cages for excreta collection. Room temperature was maintained at 70±5°C with a 12-hour light/dark cycle. The repeated-dose group received diets containing the nonlabeled test material for 14 days.

2. Dosing Solutions

The appropriate amounts of nonradiolabeled BARDAC 22 and [^{14}C]-labeled BARDAC 22 were mixed just prior to dosing. Dose solutions were delivered by gavage at a constant volume of 10 mL/kg body weight. The specific activities of the low-dose single, low-dose repeated, and high-dose single dosing solutions were approximately 24,500, 24,500, and 2500 dpm/µg BARDAC 22, respectively. Each animal received ~10-20 µCi. A diet containing 34 ppm nonlabeled BARDAC 22 (corresponded to 3.4 mg/kg/day) was prepared for the repeated-dose group. The administration of 34 ppm instead of 50 ppm (5 mg/kg/day) in the diet for 14 days should not have affected the results of the repeated-dose study. In the metabolite analysis study, specific activity of the dosing solution was 1.2 µCi/mg BARDAC 22.

In the preliminary study, the specific activity of the radiolabeled dose solution was 43,606 dpm/µg BARDAC 22.

3. Sample Collection

Urine, urine funnel washes, and feces were collected at 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours postdosing. Fecal samples were collected at 24, 48, and 72 hours postdosing for the metabolite analysis study. Urine was freeze-trapped to avoid oxidation, evaporation, and bacterial degradation. After

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animals were sacrificed on Day 7 postdosing, the following tissues were collected: gastrointestinal tract and its contents, liver, fat, kidneys, bone, heart, lungs, brain, gonads, muscle, pancreas, spleen, adrenal gland, thyroid gland, and residual carcass. Cage rinse and blood were collected at the end of the study.

Radioactivity in urine and cage washings was analyzed, in duplicate, by liquid scintillation counting (LSC) using a Beckman LS 3801, Beckman Instrument, Fullerton, CA. Feces and large tissues were homogenized, if necessary, and combusted in a Harvey Sample Oxidizer, Model OX300, prior to LSC. Whole blood and tissues were combusted prior to LSC. Methods for statistical analyses were limited to the calculation of means.

Expired CO_2 was collected in ethanolamine:cellusolve (2:1) at 2, 4, 6, 8, and 24 hours postdosing. The CO_2 trapping solution was mixed with Carbon 14 Cocktail (Harvey Instrument, Hillsdale, NJ) before counting. Urine and feces were collected at same time intervals as the expired air in order to reduce the chance of any volatilization of ^{14}C. Urine was freeze-trapped for the same reason. Urine and feces were later discarded.

4. Metabolite Analysis

Day 1 or Day 2 fecal samples were pooled by sex and dose level. Fecal samples were homogenized, centrifuged and then extracted with methanol (3x). The methanol extracts contained most of the radioactivity and were concentrated and then loaded on a silica gel column using chloroform/methanol solvent system for initial isolation. The collected radioactive fractions were further purified by thin-layer chromatography (TLC) and then high-performance liquid chromatography (HPLC). Mass spectrometry (MS) was conducted on the isolated samples.

5. Regulatory Compliances

Flagging statements were signed on January 12, 1990 (Report 1) and December 22, 1989 (Report 2). The quality assurance statements and the statements of compliance with Good Laboratory Practices were signed on December 11, 1989 and January 4, 1990, respectively, for Report 1 and on May 1, 1989 and December 22, 1989, respectively, for Report 2.

C. REPORTED RESULTS

1. Preliminary $^{14}CO_2$ Study

In the preliminary study, 0.045-0.053% of the administered radioactivity was expired in male and female rats dosed with 4.7-4.8 mg/kg [^{14}C]-BARDAC 22; the majority was detected during 0-4 hours postdosing.

2. Elimination and Recovery

At 7 days postdosing, total mean recovery of radioactivity was 90.82-100.94% of the administered dose for all dose groups (Table 1). Nearly all of the recovered radioactivity was found in the feces

(89.11-99.46% of the dose) and only a small amount in the urine (1.17-2.36% of the dose). Fecal elimination peaked at 8-36 hours postdosing for the single and repeated low-dose animals. In the high-dose animals, most of the fecal elimination occurred from 8 hours to 72 hours postdosing.

3. Tissue Distribution

At 7 days postdosing, 0.01-0.30% of the administered radioactivity was recovered in tissues, including carcass, for all dose groups. All individual tissues or organs contained negligible amounts of radioactivity; 0-0.05% of the dose (0-6.674 ppm). The carcass contained 0-0.17% of the dose.

4. Metabolism

After three methanol extractions, the unextracted residues in all fecal samples constituted <10% of total residues present in the samples. At least 60% of the radioactivity was recovered after silica gel elution. Several attempts were made to improve TLC/HPLC isolation methods, but the investigators were unable to get better separation and/or quantitation.

Preparative TLC isolated 4 radioactive bands; one was the unchanged parent compound which represented most of the radioactivity (Table 2). The metabolites were more polar than the parent compound. Because two of the bands were eluted closely (bands B1 and B2), they were pooled together for separation by HPLC. Structures of A, B1, and B2 metabolites were further analyzed by MS. Metabolite B1 (molecular ion at m/z 356) appeared to have a hydroxyl group on the terminal carbon and a ketone on the adjoining carbon. Metabolite B2 (m/z 342) appeared to be hydroxylated on the decyl side chain. MS revealed that Band A contained two metabolites, A1 (m/z 372) and A2 (m/z 358). It was concluded that metabolite A1 has a single hydroxylation at one of the decyl chains while metabolite A2 was hydroxylated on the second carbon from the end of the decyl chain. Slightly higher amounts of the parent compound were found in the low-dose groups compared to the high-dose group, as well as higher amounts in females than males for all dose groups (Table 1). Slightly higher amounts of metabolites A1 and A2 were found in the females than males.

Based on these data, metabolic pathways of BARDAC 22 were proposed (Figure 1). Oxidation of the decyl side chain appeared to be the major metabolic pathway.

D. CONCLUSIONS BASED ON REVIEWERS' DISCUSSION AND INTERPRETATION OF DATA

The study demonstrated that ^{14}C-BARDAC 22 was absorbed, distributed, metabolized and excreted, primarily in the feces, following oral administration in rats. Total recoveries of the radioactivity were high for all groups (90.82-100.94% of the administered dose). Most of the radioactivity in the dose groups were recovered in the feces (89.11-99.46% of the administered dose); however, the amounts of biliary

excretion and unabsorbed test material were not known because intravenous dosing was not conducted. Bioaccumulation of BARDAC 22 appeared to be low as indicated by <1% recovery of radioactivity in tissues at 7 days postdosing. The study results do not suggest any sex- or dose-related differences in the distribution and elimination of BARDAC 22.

Metabolism data suggest that BARDAC 22 is oxidized at the two decyl side chains to form hydroxy and hydroxyketo derivatives. MS analyses indicated that the 2 methyl substituents of BARDAC 22 remained unmodified. Furthermore, the hydroxy and/or keto functions on the decyl side chains are probably located at or near the terminal end. The authors reported slight sex- and dose-related differences in the metabolism of BARDAC 22; greater metabolism in females than males and in the low-dose groups than high-dose group. Because recovery of radioactivity following extraction with methanol and isolation from silica gel was not high, the differences in metabolism among groups are not conclusive. The authors also noted that most of the metabolism was microbial since most of the radioactivity was recovered in the feces, but this was not confirmed. Because <2.5% of the administered dose was recovered in the urine, metabolite analysis was not performed.

E. STUDY DEFICIENCIES

1. Because of the large recovery of radioactivity in the feces, an intravenous study should be conducted in order to assess the extent of oral absorption of BARDAC 22 (i.e., determine the amount of radioactivity in the feces that is a result of biliary excretion and unabsorbed test material). The high amount of parent compound in the feces suggest that a large portion of the administered dose may not have been absorbed.

2. Recovery of radioactivity in the feces following extraction and isolation procedures was not reported as percentage of administered dose; however, most of the major metabolites appeared to have been identified. The authors reported that they did attempt to improve TLC/HPLC isolation methods, but were unable to get better separation and/or quantitation.

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TABLE 1. Excretion of Radioactivity 7 Days Following Oral Administration of [^{14}C]-BARDAC 22 in Rats

Dose Group	Sex[a]	Percent of Administered Dose (mean)			
		Urine	Feces	Tissues & Carcass	Total Recovery
5 mg/kg (single)	M	1.65	89.11	0.05	90.82
	F	1.42	92.13	0.01	93.56
5 mg/kg (repeated)	M	1.19	93.88	0.02	95.09
	F	1.74	90.11	0.03	91.88
50 mg/kg (single)	M	1.17	99.46	0.30	100.94
	F	2.36	91.93	0.19	94.47

[a]5/Sex/group

Source: Report 1. Tables 34-39, pp. 63-68

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TABLE 2. Distribution of Radioactivity in Feces at 1 and 2 Days After Oral Administration of BARDAC 22

(Values Given As Percentage of Total ^{14}C in Sample)[a]

	5 mg/kg (single oral)				5 mg/kg (repeated oral)				50 mg/kg (single oral)			
	Male		Female		Male		Female		Male		Female	
Metabolites	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
Parent Compound	57.8	64.3	36.7	38.9	54.4	56.9	39.7	48.9	64.8	65.6	59.1	56.8
Metabolite A1 & A2[b]	19.1	12.6	28.8	24.7	21.1	13.7	27.1	21.9	11.7	12.0	16.7	15.6
Metabolite B1	11.1	10.9	16.8	14.0	10.8	14.5	16.2	3.5	12.2	9.8	13.3	11.2
Metabolite B2	5.0	4.2	9.6	11.0	4.9	4.6	8.9	19.9	4.1	3.4	4.6	2.9
Unidentified Radioactive TLC Fractions:												
Zone 1	1.7	2.0	1.8	1.3	2.8	1.8	1.6	1.5	3.0	0.7	2.0	1.0
Zone 5	2.3	3.1	3.0	6.7	2.6	6.0	2.5	1.6	2.3	1.9	2.6	3.5
Zone 7	2.5	2.3	3.1	4.3	3.0	2.4	3.7	2.6	1.6	2.8	1.6	3.9
Zone 8	0.4	0.5	0.3	0.1	0.5	0.2	0.3	0.2	0.3	3.9	0.2	5.3

[a]After methanol extraction

[b]Includes Metabolites A1, A2, and two unidentified minor metabolites with molecular cations at m/z 386 and 400.

Source: Report 2, Table 3, p. 23

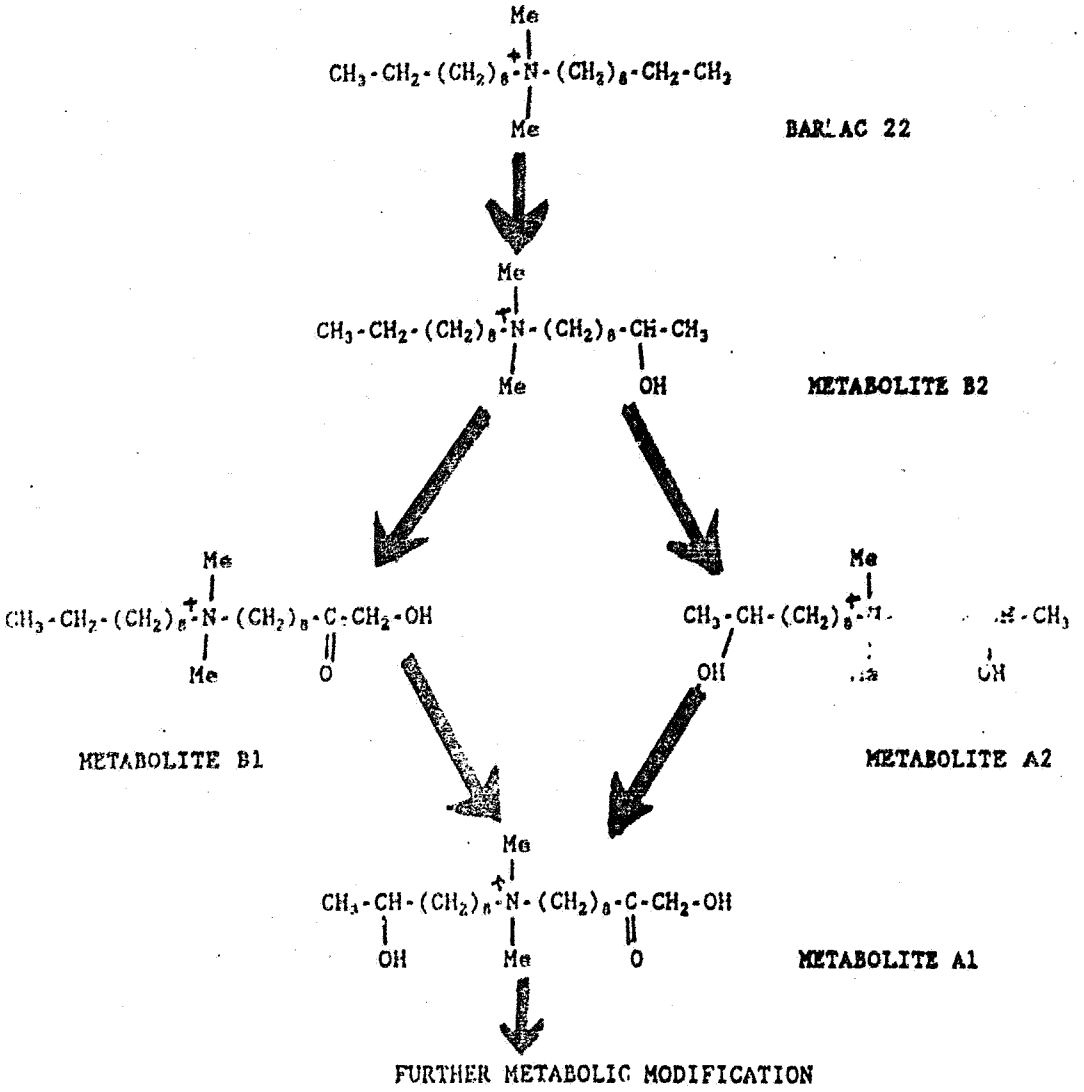

Figure 1. Proposed metabolic pathway for BARDAC 22

Source: Report 2, Figure 16, p. 39

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FINAL

DATA EVALUATION REPORT

ADBAC

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT)

Prepared for:

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

Prepared by

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

Principal Reviewer — Kristin Jacobson, MSPH — Date 11/9/93

Independent Reviewer — Nancy E. McCarroll, B.S. — Date 11/9/93

QA/QC Manager — Sharon Segal, Ph.D. — Date 11/9/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 298
Project Officer: Caroline Gordon

010689

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

EPA Reviewer: Robert Fricke, Ph.D.
Review Section IV, Toxicology Branch II
Health Effects Division (7509C)
Signature: Robert J. Fricke
Date: 18 Nov 93

EPA Section Head: Jess Rowland, M.S.
Review Section IV, Toxicology Branch II
Health Effects Division (7509C)
Signature: Jess Rowland
Date: 11/19/93

DATA EVALUATION REPORT

STUDY TYPE: Gene mutation in cultured Chinese hamster ovary cells (CHO/HGPRT) (84-2)

TEST MATERIAL: N-alkyl dimethyl benzyl ammonium chloride (ADBAC)

PC CODE: 069105

DP BARCODES: D188679, D189620

SUBMISSION NOS.: S436173

CASWELL NO.: 01[illegible]

MRID Number: 410127-01

SYNONYM(S): None provided

SPONSOR: ADBAC Quat Joint Venture/Chemical Specialties Manufacturers Assoc., Washington, D.C.

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD

TITLE OF REPORT: Mutagenicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the CHO/HGPRT Forward Mutation Assay

AUTHOR: R.R. Young

STUDY NUMBER: HLA Study No. 10238-0-435

REPORT ISSUED: January 23, 1989

CONCLUSIONS--EXECUTIVE SUMMARY:

Negative for the induction of forward gene mutations in Chinese hamster ovary (CHO) cells at the HGPRT locus in two independent assays conducted with levels ranging from 1-20 µg/mL - S9 and 1-40 µg/mL +S9. Severe cytotoxicity was demonstrated at doses ≥20 µg/mL -S9 and ≥40 µg/mL +S9.

CORE CLASSIFICATION: Acceptable. The study satisfies the Guideline requirement (84-2) for genetic effects, Category I, Gene Mutations, and is acceptable for regulatory purposes.

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MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS

1. Test Material: ADBAC

Description: Slightly viscous, clear yellow liquid
Identification number: Lot number 7293K
Purity: 80%
Receipt date: January 21, 1988
Stability: Stable in the solvent for 24 hours at 5°C
Contaminants: None listed
Solvent used: Deionized water (dH_2O)
Other provided information: The test material was stored at room temperature in the dark. Dosing solutions were prepared on the day of testing. Actual test material concentrations were determined on the primary stock solutions for each mutation assay; in addition, the 24-hour stability of the test material in the solvent was analytically verified. The report did not indicate that solutions were adjusted to 100% purity.

2. Culture Medium: Ham's Nutrient Mixture F12 containing 10% fetal bovine serum (FBS), L-glutamine and antibiotics. For the S9-activated assays, the FBS concentration was reduced to 5%.

3. Control Materials:

Negative: Culture medium (cytotoxicity assay only)

Solvent: dH_2O, at a final concentration of 10%

Positive: Nonactivation: 5-Bromo-2'-deoxyuridine (BrdU) was prepared in an unspecified solvent to yield a final concentration of 50 µg/mL.

Positive: Activation: 3-Methylcholanthrene (3-MC) was prepared in an unspecified solvent to yield a final concentration of 5 µg/mL.

4. Activation: S9 derived from Sprague-Dawley (sex not specified)

_X_ Aroclor 1254	_X_ induced	_X_ rat	_X_ liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 homogenate was purchased commercially (supplier not specified). Prior to use, CHO/HGPRT assays were conducted with two reference mutagens (benzo(a)pyrene and 3-MC) and various concentrations of S9 in order to select the optimum S9 concentration. The S9 mix was prepared as follows:

Component	Final Concentration
Phosphate buffer	2.0 mM
NADP	1.0 mM
Glucose-6-phosphate	5.0 mM
$CaCl_2$	2.0 mM
KCl	6.6 mM
$MgCl_2$	2.0 mM
S9 homogenate	20.0 µL/mL (2%)

5. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
_X_ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Yes.
Periodically "cleansed" against high spontaneous background? Yes.

6. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

_X_ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) 4 µg/mL 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

7. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Ten doses (1, 2, 5, 10, 20, 50, 100, 200, 500, and 5000 µg/mL) were evaluated in the presence and absence of S9 activation, using triplicate cultures.

(b) Mutation assay: Two independent assays were conducted with single cultures in the presence or absence of S9 activation, as follows:

(1) Nonactivated conditions:

Initial trial: 1, 5, 10, 13, 16, 20, 25, 35, 50, and 65 µg/mL. Cells treated with the five levels ≤16 µg/mL were plated for selection.

Repeat trial: 1, 5, 10, 12, 14, 16, 18, 20, and 24 µg/mL. Cells treated with the seven levels between 5 and 20 µg/mL were plated for selection.

(2) S9-activated conditions:

Initial trial: 1, 5, 10, 20, 30, 40, 50, 65, 85 and 100 µg/mL. Cells treated with the five lowest dose levels (i.e., ≤30 µg/mL) were plated for selection.

Repeat trial: 10, 20, 22, 24, 26, 28, 30, 40 and 50 µg/mL. Cells treated with the seven levels between 20 and 40 µg/mL were plated for selection.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells were exposed to the test compound, solvent or positive control for: 4 hours (nonactivated or S9-activated conditions)

(b) After washing, cells were cultured for 7 days (expression period) before cell selection.

(c) After expression, 2×10^5 cells/dish (12 dishes/culture) were cultured for 7-10 days in selection medium to determine numbers of mutants; 200 cells/dish (3 dishes/culture) were cultured for 7-10 days in nonselection medium to determine cloning efficiency (CE)

2. Statistical Methods: Mutation frequency (MF) data were analyzed for statistical significance ($p \leq 0.05$ and 0.01) using a two-tailed binomial approximation of a Poisson distribution, using the tables of Kastenbaum and Bowman (1970).[1]

[1]Kastenbaum, M.A. and Bowman, K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat Res 9: 527-549.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if the following conditions were met: (1) the CE in the vehicle controls was between 50-115%; (2) the MF in the vehicle controls was $\leq 15 \times 10^{-6}$; (3) the MF of the positive control was significantly ($p \leq 0.01$) higher than the solvent control; (4) the test material was tested either to the maximum concentration, to a concentration approximately twice that of the test material's solubility limit in the culture medium, to a concentration that reduced relative survival (RS) to 85-90%, or to 75% of a highly cytotoxic concentration; (5) the MF at each dose level was based on the counts from at least eight of twelve mutant selection dishes and from 2 of 3 cloning efficiency dishes; and (6) at least three dose levels were evaluated for induction of mutations.

(b) Positive response: The test material was considered positive if a significant ($p \leq 0.05$), dose-related increase in the MF above 15×10^{-6} was observed, or if there was a >2-fold increase at a single dose level near the cytotoxicity limit. Smaller increases must have been reproduced in a confirmatory assay.

C. REPORTED RESULTS:

1. Test Material Solubility/Solution Preparation: The test material was soluble in dH_2O up to 50 mg/mL but a white precipitate was reported in culture medium containing ≥50 µg/mL. Addition of the test material to the culture medium did not alter the pH. The study author stated that all primary stock solutions were prepared with plastic pipets and tubes. For the mutation assays, test solutions were prepared with glass pipets and volumetric flasks.

2. Preliminary Cytotoxicity Test: The nonactivated test material was severely cytotoxic (i.e., no colonies surviving) at levels ≥20 µg/mL. Relative survival was reduced to 53.4% at 10 µg/mL; lower doses (1-5 µg/mL) did not have an appreciable effect on survival, with RS ≥89%. Under S9-activated conditions, the test material was slightly less cytotoxic; doses ≤20 µg/mL did not have an adverse effect on RS, whereas no cells survived exposure to levels ≥50 µg/mL.

Based on these findings, doses representing a cytotoxicity range of approximately 0-90% (1-65 µg/mL -S9 and 12-100 µg/mL +S9) were selected for the initial mutation assays.

3. Mutation Assays:

Nonactivated conditions: Representative results from the two nonactivated assays are presented in Table 1. In agreement with the findings of the preliminary cytotoxicity study, levels ≥20 µg/mL -S9

in the initial nonactivated trial were severely cytotoxic. RS for the remaining concentrations was dose related and ranged from 8.6% at 15 µg/mL to >100% at ≤5 µg/mL. There were, however, no appreciable increases in the MF at any dose. In the repeat trial, a narrower range of doses (5-24 µg/mL) was evaluated. Survival was generally dose related and RS in cultures exposed to 20 or 24 µg/mL was <5%. The study author reported that MFs for the 16, 18, and 20 µg/mL cultures were significantly elevated over the solvent control MF; these elevations were not considered biologically meaningful, however, because they were within the expected spontaneous range of $0\text{-}15\times10^{-6}$ and similar elevations had not occurred in the initial trial.

S9-activated conditions: Table 2 presents representative data from the two S9-activated assays. Results from both trials were in good agreement and indicated that levels ≥40 µg/mL were severely cytotoxic; there were also no increases in the MF at any dose.

In contrast, the positive controls (nonactivated 50 µg/mL BrdU and S9-activated 5 µg/mL 3-MC) induced significant ($p\leq0.01$) increases in the MFs in both trials.

4. Analytical Determinations: Using spectrophotometry, duplicate samples of the test material primary stock solutions for each mutation assay were analyzed for actual test material concentration. All samples were within 7% of the respective target concentrations. In addition, the 24-hour stability of the test material (at 5°C) was verified for solutions containing 1.0 and 100 µg/mL test material.

Based on the overall findings, the study author concluded that ADBAC was not mutagenic in the CHO/HGPRT forward gene mutation assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that ADBAC was tested over a range of concentrations that included severely cytotoxic doses (≥20 µg/mL -S9 and ≥40 µg/mL +S9) but failed to induce a mutagenic response. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the nonactivated (50 µg/mL BrdU) and S9-activated (5 µg/mL 3-MC) positive controls. We conclude, therefore, that ADBAC is not a mutagen in this *in vitro* mammalian cell gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated January 23, 1989.)

CORE CLASSIFICATION: Acceptable. The study satisfies the Guideline requirement (84-2) for genetic effects, Category I, Gene Mutations, and is acceptable for regulatory purposes.

Table 1. Representative Results of the Nonactivated Chinese Hamster Ovary (CHO) Cell Forward Gene Mutation Assays with ADBAC

Substance	Dose	Relative Percent Cloning Efficiency (after treatment)[a]	Number of Mutant Colonies	Absolute Percent Cloning Efficiency (at selection)[a]	Mutation Frequency x10^{-6}[b]
Solvent Control[c]					
Deionized water	18I	100.0 (91.5)[d]	8	0.92	3.6
		100.0 (100.0)[e]	14	0.96	6.2
Positive Control					
5-Bromo-2'-deoxy-uridine	50 µg/mL	81.0[d]	269	0.82	133.3[f]
		64.2[e]	259	0.95	123.6[f]
Test Material					
ADBAC	13 µg/mL[g]	45.2[d]	7	0.93	3.1
	14 µg/mL[h]	8.6	3	0.98	1.3
	14 µg/mL[g]	33.7[e]	9	1.05	3.4
	15 µg/mL	19.2	26	0.84	12.9
	18 µg/mL	13.4	25	0.91	11.5
	20 µg/mL[g]	4.0	33	0.94	14.6

[a]Based on the results of single cultures per treatment or positive control group and duplicate cultures in the solvent control group. Values in () are absolute survival; calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{(\text{No. of Dishes, 12})(\text{No. of Cells Plated, } 2\text{x}10^5)(\text{Cloning Efficiency})}$; calculated by our reviewers.

[c]Solvent control values represent the average value from duplicate cultures; calculated by our reviewers.
[d]Results from the initial trial
[e]Results from the repeat trial
[f]Findings for lower doses (1, 5, and 10 µg/mL -- initial trial; 5, 10, and 12 µg/mL -- repeat trial) did not suggest a mutagenic response. Higher doses (20, 25, 35, 50, and 65 µg/mL -- initial trial; 24 µg/mL -- repeat trial) were severely cytotoxic and were, therefore, not plated for selection.

[g]Significant ($p \leq 0.01$) by 2-tailed binomial test (i.e., Kastenbaum-Bowman tables) and higher than the acceptable background range of <13x10^{-6}

Note: Data were extracted from the study report, pp. 29-..

Table 1

MAMMALIAN CELLS IN CULTURE GENE MUTATION

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FINAL

DATA EVALUATION REPORT

ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE (ADBAC)

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes (84-4)

Prepared for:

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

Prepared by:

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

Principal Reviewer _____ Dean Walton, Ph.D. Date 11/1/93

Independent Reviewer _____ Nancy E. McCarroll, B.S. Date 11/1/93

QA/QC Manager _____ Sharon Segal, Ph.D. Date 11/11/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 299
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY
UDS

MUTAGENICITY STUDIES

EPA Reviewer: Robert Fricke, Ph.D. Signature: Robert F. Fricke
Review Section IV, Date: 18 Nov 93
Toxicology Branch IV/HED H7509C
EPA Section Head: Jess Rowland, M.S. Signature:
Review Section IV,
Toxicology Branch IV/HED H7509C Date: 11/19/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Unscheduled DNA synthesis assay in primary rat hepatocytes (84-4)

CHEMICAL: Alkyl dimethyl benzyl ammonium chloride (ADBAC)

DP BARCODES: D188679 Submission No.: S436173
D189620

Caswell No.: 016E

P.C. CODE: 069105

MRID Number: 422908-01

SYNONYM/CAS No.: ADBAC

SPONSOR: ADBAC Quat Joint Venture/Chemical Specialties Manufactures Association, Washington, DC

TESTING FACILITY: Hazleton Washington, Inc., Kensington, MD and Vienna, VA

TITLE OF REPORT: Genotoxicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Assay for Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures

AUTHORS: M.E. McKeon and M. Phil

STUDY NUMBER: 14778-0-447

REPORT ISSUED: April 15, 1992

CONCLUSIONS-EXECUTIVE SUMMARY: Negative for inducing unscheduled DNA synthesis (UDS) in primary rat hepatocytes treated with the test material at doses up to 6.46 µg/mL. Higher doses (≥7.00 µg/mL) were severely cytotoxic.

CORE CLASSIFICATION: Acceptable. This study satisfies guideline requirements for an in vitro (UDS) mutagenicity study (84-4) and is acceptable for regulatory purposes.

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A. MATERIALS:

1. Test Material: ADBAC

Description: Clear, yellow liquid
Lot numbers: 7293K; Code No. 12347
Purity: 80%
Receipt date: September 25, 1991
Stability: Not reported
Contaminants: Not reported
Solvent used: Deionized water (dH_2O)
Other provided information: All solutions were prepared fresh and were analyzed to verify target concentrations (see Section C: Reported Results).

2. Indicator Cells: Rat hepatocytes, harvested from a 190.4-g adult female rat (Strain: Fischer-344 (F344/NHSD) purchased from Harlan Sprague Dawley Inc.

3. Control Substances:

- The solvent control was sterile dH_2O at a final concentration of 10% in the media.
- The positive control was 4.48×10^{-7} M (0.10 µg/mL) acetylaminofluorene (2-AAF) prepared in dimethyl sulfoxide (DMSO). The concentration of DMSO did not exceed 1% in the media.

4. Medium: William's Medium E (WME) contained 2 mM L-glutamine and antibiotics. WME+: as above with 10% fetal bovine serum. WMC: WME + 100 units/mL of collagenase.

5. Test Compound Concentrations Used: Concentrations were based on previously published data.[1] Fourteen doses ranging from 0.538 µg/mL to 11.8 µg/mL were evaluated. Cells exposed to 0.538, 1.08, 2.15, 4.31, 5.38, or 6.46 µg/mL were scored.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion technique: The liver was perfused in situ with Ca^{++}/Mg^{++}-free Hank's balanced salts containing 0.5 mM EGTA and 50 mM HEPES buffer solution (pH 7.2) for ~4 minutes and with WMC for 10 minutes. The liver was excised, moved to a culture dish containing WMC and mechanically dispersed to release the hepatocytes.

[1] Cifone, M.A. (1989). Mutagenicity test on alkyl dimethyl benzyl ammonium chloride (ADBAC) in the rat primary hepatocyte unscheduled DNA synthesis assay. MLA Study 10238-0-447.

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(b) Hepatocyte harvest/culture preparation: Recovered cells were centrifuged, resuspended in WME+, counted, and ~0.5x10^6 cells/3 mL were dispensed into culture dishes containing plastic coverslips. The cultures were placed in a humidified, 37°C, 5% CO_2 incubator for a 2.0-hour attachment period. Unattached cells were removed; viable cells were refed and established as monolayer cultures.

2. UDS Assay:

(a) Treatment: Five replicate monolayer cultures were exposed to the selective doses of the test material, negative or positive controls (2-AAF) for 18.8 hours in WME containing 10-11.1 μCi/mL [^{3}H]-thymidine (48 Ci/mmole). Treated monolayers were washed twice with WME; two of the five replicates for each treatment group were used to determine cytotoxicity. These cultures were refed, reincubated and monitored for cytotoxicity at 20.5 hours posttreatment by trypan blue exclusion.

(b) UDS slide preparation: The remaining cultures were washed with WME containing 1 mM thymidine. Treated hepatocytes, attached to coverslips, were swollen with 1% sodium citrate and fixed in ethanol:acetic acid (3:1). The coverslips were mounted onto slides and each slide was dipped in NTB2 photographic emulsion (Kodak) and dried.

(c) Preparation of autoradiographs/grain development: Slides were exposed for 7 days at 4°C in light-proof boxes containing desiccant, developed with Kodak D-19, fixed and stained with Williams' modified hematoxylin-eosin. Slides were coded prior to analysis.

(d) Grain counting: The nuclear and cytoplasmic grains of 150 cells (50 cells/coverslip) per treatment were counted. The net nuclear grain counts were quantitated by subtracting the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus from the nuclear grain count. Cells with blackened nuclei were not scored.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if: (1) hepatocytes recovered from the perfusion step and monolayer cultures used for the assay reached the ≥70% viability level; (2) the negative control had a net nuclear grain count of -5.0 to 1.0 and no more than 10% of the nuclei had ≥5 net grains; (3) the positive control demonstrated the sensitivity of the test system to detect UDS; (4) data were obtained from at least two replicate cultures/dose; and (5) the highest of at least 6 doses was cytotoxic, limited by solubility, or reached the maximum recommended dose for this assay (5000 μg/mL).

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(b) Positive response: The test material was considered positive if: (1) the increase in the mean nuclear grain count was ≥5 grains/nucleus over the negative control value; and (2) the percent of nuclei with ≥5 grains exceeded 10% of the negative control population.

4. Statistical Methods: The data were not analyzed for statistical significance.

C. REPORTED RESULTS:

1. UDS Assay: Six doses, ranging from 0.538 µg/mL to 8.61 µg/mL of the test material were examined in a parallel cytotoxicity and UDS assay. The data indicated that doses ≥7.00 µg/mL either caused marked reductions in cell viability or adverse effects on cellular morphology. Accordingly, hepatocytes treated with 0.538, 1.08, 2.15, 4.31, 5.38, or 6.46 µg/mL of the test material were scored for the incorporation of tritiated thymidine. Representative findings presented in Table 1 show that the selected doses of ADBAC did not induce a genotoxic effect. At the highest dose scored, 6.46 µg/mL ADBAC, the percentage of cells with ≥5 mean nuclear grains was slightly elevated. However, the percentage of cells with ≥5 net nuclear grains (5.00%) was well within the historical background range (1.3-8.7%) of the reporting laboratory (see Study Report p. 50). By contrast, marked increases in the net nuclear grain counts and the percentage of cells in repair were observed in hepatocytes exposed to the positive control (0.10 µg/mL 2-AAF). Cell viability for this group was 94.5%.

2. Analytical Determi... Stock solutions of the test material ranging from 5.382 µ... to 118.4 µg/mL were prepared using volumetric flasks an... pipets. Each solution was analyzed to verify target levels, and results of the analysis revealed that all solutions were accurately prepared and were within ±4% of the target level.

Based on the overall results, the study author concluded that ADBAC was not genotoxic in this test system and that these findings confirmed the results of an earlier UDS assay conducted with male rat hepatocytes.[2]

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We agree with the study author's conclusions that ADBAC did not induce genotoxic effects at the concentrations tested in this rat hepatocyte DNA repair assay. We further assess that the material was assayed over an appropriate range that included cytotoxic levels; therefore, the assay provided valid evidence of a negative response in this test system. In addition, the sensitivity of the test system to detect UDS was adequately demonstrated by the results obtained with the positive control (0.100 µg/mL 2-AAF).

[2]Clifone, M.A. (1989). Mutagenicity test on alkyl dimethyl benzyl ammonium chloride (ADBAC) in the rat primary hepatocyte unscheduled DNA synthesis assay. MLA Study 10233-0-447.

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TABLE 1. Representative Results of the Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assay with ADBAC

Treatment	Dose	Number of Cells Scored/ Group	Net Nuclear Grains[a]	Percent of Cells in Repair (≥5 Net Nuclear Grains)	Percent Survival 20 Hours
Solvent Control					
Deionized water	10X	150	-1.29±0.58	2.00	100.0
Positive Control					
2-Acetylaminofluorene	0.10 µg/mL	100	18.40±1.07	96.00	94.5
Test Material					
ADBAC	5.38 µg/mL[b]	150	-1.99±0.80	0.67	79.2
	6.46 µg/mL[c]	100	-0.80±0.37	5.00	<[illegible].7

[a]Mean and standard deviations of net nuclear grain counts for 150 cells (solvent control and 5.38 µg/mL treatment group) and 100 cells (positive control and 6.46 µg/mL treatment group); 50 cells/slide were analyzed.
[b]Results from lower doses (0.538, 1.08, 2.15, or 4.31 µg/mL) did not suggest a genotoxic effect.
[c]Higher doses (7.00, 7.54, 8.07 or 8.61 µg/mL) were cytotoxic and were not scored.

Note: Data were extracted from the study report pp. 20-21.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated April 15, 1992.)

CORE CLASSIFICATION: Acceptable. The study satisfies guideline requirements for an in vitro (UDS) mutagenicity study (84-4) and is acceptable for regulatory purposes.

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FINAL

DATA EVALUATION REPORT

ADBAC

Study Type: Mutagenicity: Micronucleus Assay with Mice

Prepared for:

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

Prepared by:

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

Principal Reviewer	Lynne T. Haber, Ph.D.	Date 11/15/93.
Independent Reviewer	Nancy E. McCarroll, B.S.	Date 11/15/93
QA/QC Manager	Sharon Segal, Ph.D.	Date 11/15/93

Contract Number: 68D10075
Work Assignment Number: 2-101
Clement Number: 292
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY MICRONUCLEUS

MUTAGENICITY STUDIES

EPA Reviewer: Robert Fricke, Ph.D. Signature: Robert F. Fricke
Review Section IV, Date: 11/19/93
Toxicology Branch II/HED (H7509C)
EPA Section Head: Jess Rowland, M.S. Signature:
Review Section IV
Toxicology Branch II/HED (H7509C) Date: 11/19/93.

DATA EVALUATION REPORT

CHEMICAL: Hyamine 3500

DP BARCODES: D188679, D189620

SUBMISSION NUMBERS: S436173

PC CODE: 069105

CASWELL NUMBER: 016E

STUDY TYPE: Mutagenicity: Micronucleus assay with mice

MRID Number: 403111-01

SYNONYMS/CAS No.: Ityamine 3500, ADBAC, Alkyl dimethyl benzyl ammonium chloride

SPONSOR: Lonza AG, Basle, Switzerland and Fair Lawn, NJ/Chemical Specialties Manufacturers Association, Washington, DC

TESTING FACILITY: Scantox Laboratories, Ltd., Skensved, Denmark

TITLE OF REPORT: Series 84-2 Assessment of the Mutagenic Activity of Ityamine 3500 in the Mouse Nucleus Test

AUTHOR: Kallersen, Th.

STUDY NUMBER: 1075.3

REPORT ISSUED: December 16, 1985

CONCLUSIONS--EXECUTIVE SUMMARY: Negative in the the mouse micronucleus assay conducted with a single oral gavage administration of 400 mg/kg Hyamine 3500 and harvests 24, 48, and 72 hour postexposure. One treated male died. The test material was cytotoxic to the target tissue, as shown by the reduced proportion of PCEs at all sacrifice times, especially with the males. However, an adequate explanation for the adjustment of the pH of the drinking water to 2.5 should be provided.

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CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirement (§84-2) for genetic effects Category II, Structural Chromosome Aberrations and is not acceptable for regulatory purposes. The study can be upgraded if the requested information is provided.

A. MATERIALS:

1. Test Material: Hyamine 3500

Description: None provided
Identification number: Batch number L-5383
Purity: 80.2%
Receipt date: Sent to the performing laboratory September 3, 1985
Stability: Not reported
Contaminants: Free amine, 0.2%; aminehydrochloride, 0.1%; ash, <0.1%
Vehicle used: Distilled water (DH_2O)
Other provided information: Neither the storage conditions nor the frequency of dosing solution preparation were reported. Analytical determination to verify the dosing concentration was not performed.

2. Control Materials:

Negative: None

Vehicle/final concentration/route of administration: DH_2O/10 ml/kg/ oral gavage

Positive/final dose(s)/route of administration: Cyclophosphamide (CP) was dissolved in DH_2O and delivered at a dose of 30 mg/kg by oral gavage.

3. Test Compound:

Route of administration: Oral gavage

Volume of test substance administered: 10 ml/kg; the report did not state whether the volume was based on individual body weights.

Dose levels used:

(a) Acute dose range-finding study: "Various" concentrations, including 400 mg/kg

(b) Micronucleus assay: 400 mg/kg

4. Test Animals:

(a) Species: Mouse Strain: NMRI SPF Age: 6-7 weeks (at dosing) Weight range at dosing: 25-30 g (males and females) Sex: Males and females Source: Bred at Scantox Laboratories, Skensved, Denmark; breeders purchased from Bomholtgard Ltd. (location not reported)

(b) Number of animals used per dose:

Range-finding study: Not specified; the report stated that "a few" were treated.

Micronucleus assay:

- Treatment group: 15 males 15 females
- Positive control: 5 males 5 females
- Vehicle control: 5 males 5 females

(c) Were test animals properly maintained? Not clear. The pH of the drinking water was adjusted to 2.5 with hydrochloric acid. No rationale for this procedure was provided. Animals were not fasted prior to the administration of the test material.

B. TEST PERFORMANCE:

1. Range-finding Assay: "A few" mice (sex not specified) were administered the test material in water at "various concentrations." Mortality was assessed. Bone marrow smears were prepared and the number of polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were determined. Further details were not provided.

2. Micronucleus Assay:

(a) Treatment and sampling times:

1. Test compound:
Dosing: X once ____ twice (24 hours apart)
____ other (describe):

Sampling (after last dose): ____ 6 hours ____ 12 hours
X 24 hours X 48 hours X 72 hours

2. Vehicle control:
Dosing: X once ____ twice (24 hours apart)
____ other (describe):

Sampling (after last dose): ____ 6 hours ____ 12 hours
X 24 hours ____ 48 hours ____ 72 hours
____ other (describe):

3. Positive control:
Dosing: X once ____ twice (24 hours apart)
____ other (describe):

Sampling (after last dose):
X 24 hours ____ 48 hours ____ 72 hours

3. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Number observed while scoring 1000 erythrocytes

4. Details of Cell Harvest and Slide Preparation: At 24, 48, and 72 hours after the administration of the test material, the appropriate groups of animals were sacrificed by cervical dislocation. Animals in the solvent and positive control groups were sacrificed 24 hours postexposure. The bone marrow from both femurs of each animal was aspirated, mixed with fetal bovine serum (FBS) and centrifuged. The supernatant was removed, and cell pellets were mixed with residual FBS and spread onto slides. Slides were fixed in methanol, stained with May-Grünwald/Giemsa and coded. The slides were scored for micronuclei in polychromatic erythrocytes (MPEs) and micronuclei in normochromatic erythrocytes and the ratio of PCEs to NCEs was determined.

5. Statistical Methods: To determine whether the results from any cell harvest time were significantly different from vehicle controls, the frequency of MPEs was transformed according to Blom's method,[1] and a one-way analysis of variance (ANOVA) was performed. ANOVA was also performed on the percent of PCEs. The threshold of significance was not reported.

6. Evaluation Criteria: No criteria were provided to evaluate assay validity, a positive response, or the biological significance of the findings.

C. REPORTED RESULTS:

1. Acute Dose Range-finding Study: Mortality at unspecified doses >400 mg/kg was reported to be "too high," and the ratio of PCEs to NCEs was reduced at 400 mg/kg. No further details were furnished. Based on these results, 400 mg/kg was selected as the maximum tolerated dose for the micronucleus assay, with sampling at 24, 48, and 72 hours.

2. Micronucleus Assay: One male treated with Hyamine 3500 was found dead on the third day posttreatment. Clinical signs, if any, were not reported. The proportion of PCEs in the bone marrow harvested from the test group was significantly reduced at all sacrifice times and the effect appeared to be slightly more pronounced in the males. This data showing that the test material was cytotoxic to the target organ was consistent with similar findings reported for the acute dose range-finding study. There was, however, no evidence of genotoxicity

[1]Blom, G. (1958). Statistical estimates and transformed beta variables. New York: John Wiley and Sons, Inc.

at any harvest time (24, 48, or 72 hours) in either the males or females. In contrast, the positive control (CP at 30 mg/kg) induced a marked increase in the frequency of MPEs in bone marrow cells harvested from both sexes at 24 hours. Results are presented in Table 1.

Based on the overall findings, the study author concluded that Hyamine 3500 was not genotoxic in this in vivo mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that Hyamine 3500 was adequately tested to a level that caused reproducible cytotoxicity but failed to induce a genotoxic response in either males or females 24, 48, or 72 hours postexposure. Additionally, the sensitivity of the test system to detect genotoxicity was adequately demonstrated by the results obtained with the positive control. Although we conclude that the study provided adequate evidence that the test material was negative in the test system, a reasonable explanation for the adjustment of the pH of the drinking water to 7.5 should be provided. The study is, therefore classified as unacceptable, but it can be upgraded if the aforementioned explanation is provided.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Not known. (The submitter included a signed, but undated, statement declaring that it was not known whether the study was conducted in accordance with GLPs. A quality assurance statement prepared by the performing laboratory, signed and dated December 16, 1985, stated that the report accurately described the methods and results of the study.)

F. APPENDIX: Appendix A, Certificate of Analysis, study p. 15.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirement (§84-2) for genetic effects Category II, Structural Chromosome Aberrations and is not acceptable for regulatory purposes. The study can be upgraded if the requested information is provided.

TABLE 1. Results of the Micronucleus Assay in Mice with Hyamine 3500

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group[b]	Average Percent MPEs[c]	Average Percent PCEs[d]
Vehicle Control								
Distilled water	10 ml	24	M	5	5000	6	0.12	46
			F	5	5000	(10)	0.08 (0.10)	46 (46)
Positive Control								
Cyclophosphamide	30 mg	24	M	5	5000	2	4.7	36
		24	F	5	5000	(11)	4.1 (4.4)	35 (36)
Test Material								
Hyamine 3500	400 mg	24	M	5	5000	4	0.08	36
			F	5	5000	4 (8)	0.08 (0.08)	43 (40)**
		48	M	5	5000	6	0.12	40
			F	5	5000	5 (11)	0.10 (0.11)	43 (41)*
		72	M	4*	4000	5	0.13	34
			F	5	5000	4 (9)	0.08 (0.10)	41 (38)**

[a]Time after compound administration by oral gavage

[b]Values in parentheses are the combined total for the male and female dosing groups.

[c]Calculated by our reviewers; values in parentheses are the combined averages for the male and female dosing groups.

[d]Percent PCEs = $\frac{\text{PCEs}}{\text{PCEs} + \text{NCEs}}$

*One male in the 72-hour test group was found dead on day 3 postdosing.

Abbreviations used: PCE = polychromatic erythrocyte; MPE = micronucleated polychromatic erythrocyte; NCE = normochromatic erythrocyte

*Significantly different ($p \leq 0.05$) from the vehicle control by ANOVA
**Significantly different ($p \leq 0.01$) from the vehicle control by ANOVA

Note: Data were extracted from the study report, pp. 12-14.

MICRONUCLEUS

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25, 1/82

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 18 Nov 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/19/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 010689

STUDY TYPE: Metabolism - Rat (§85-1)

DP BARCODES: D188679, D189620 **SUBMISSION NO.:** S436173

P.C. CODE: 069105 **CASWELL NO.:** 016E

MRID NO.: 409907-01

TEST MATERIAL: ADBAC

SYNONYMS: Alkyl dimethyl benzyl ammonium chloride

STUDY NUMBER: P01359

SPONSOR: ADBAC QUAT Joint Venture/Chemical Specialist Manufacturers Association, Washington, D.C.

TESTING FACILITY: Biological Test Center, Irvine, CA

TITLE OF REPORT: Absorption, distribution, metabolism and excretion of alkyl dimethyl benzyl ammonium chloride (ADBAC) in the rat

AUTHOR: S. Selim

REPORT ISSUED: 26 January 1989

CONCLUSIONS: The pharmacokinetic profile of ADBAC was studied in male and female rats orally gavaged with low (10 mg/kg), high (50 mg/kg) or repeated low dose (100 ppm in diet for 14 days followed by single dose at 10 mg/kg) of test compound, additionally an i.v. low (10 mg/kg) dose study was also performed. Fecal elimination accounted for > 90%, while 5.8 to 7.8% appeared in the urine. For i.v. dosing 20 to 30% appeared in the urine with 44 to 55% in the feces. Tissue accumulation of orally-dosed animals negligible (< 1% of administered dose), while the carcasses and tissues of i.v. dosed animals retained 33.4% (males) and 35.8% (females) of the administered dose. Identification of metabolites were not performed in this study.

CORE CLASSIFICATION: Supplementary. This study does not satisfy guideline requirements (§85-1) for a metabolism study in rats and is not acceptable for regulatory purposes. The study may be upgraded to guideline if metabolic profile data are submitted and judged to be acceptable.

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010689

I. OBJECTIVE: The objective of this study was to evaluate the absorption and elimination of orally and intravenously administered test compound. The metabolic profile was not evaluated in this study, but as stated in the study, it will be the subject of another report.

II. MATERIALS

A. Test Compound and Dosing Solutions

1. Radiolabeled material: ADBAC was uniformly labeled on benzene ring with ^{14}C. ^{14}C-ADBAC had a radiochemical purity of > 99.4% and a specific activity of 24 mCi/mmole. The lot number was not given in the study.

2. Nonradioactive material: Unlabeled ADBAC (Lot No. 05-6K) contained 30% active ingredient.

3. Dosing solutions: An appropriate amount of test material was dissolved in water and administered either orally at 10 ml/kg or intravenously at 2.5 ml/kg.

B. Test Animals: Species: Rat Strain: Sprague-Dawley CD (SE) Br Age: 6 weeks Weight (g): see II.A.1 and 2 Source: Charles River Breeding Laboratories, Inc., Portage, MI. Housing: Individually in suspended cages Feed: Wayne Rat Chow Water: Tap water, *ad libitum* Environment: Temperature, 74 ± 0.5 °F; Humidity, Not given; Light cycle, 12 hr light/12 hr dark

III. METHODS

A. Preliminary Experiments

1. Preliminary Expired $^{14}CO_2$ Experiment: Animals (2/sex) had mean body weights of 170g (males) and 124g (females). Following an overnight (18 hours) fast, animals were given an oral administration of ^{14}C-ADBAC at 10 mg/kg (15.4 µCi, males; 11.1 µCi, females) and immediately placed in glass metabolism cages. Expired CO_2 was trapped in ethanolamine:cellusolve (2:1) and assayed at 2, 4, 6, 8, 12, and 24 hour intervals. To preclude contamination of the trapping solution by labeled volatile components in the urine and feces, the excreta were freeze trapped in the metabolism cages and discarded. Five ml aliquots of the trapping solution were assayed for radioactivity.

2. Preliminary Blood Level Experiment: In a preliminary blood level experiment, animals were dosed orally with 10 mg/kg ^{14}C-ADBAC (15.5 µCi, males; 11.7 µCi, females). Blood samples (100 µl) were collected from the tail vein at 15, 30 and 45 min and 1, 2, 3, 4, 6, 8, 12, and 24 hours.

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B. Definitive Experiments

1. In the definitive experiments, animals (5/sex/group) were randomly assigned to low dose (10 mg/kg, ^{14}C-ADBAC), repeat low dose (14 days at 100 ppm in diet, 10 mg/kg, ^{14}C-ADBAC on Day 15), high dose (50 mg/kg, ^{14}C-ADBAC) and i.v. low dose (10 mg/kg, ^{14}C-ADBAC) study groups. Mean body weights at time of dosing ranged from 269 to 342g for males and 161 to 208g for females. After administration of labeled test material (10.1 to 13.9 µCi) urine and feces were collected at selected intervals. Seven days after dosing, animals were anesthetized and exsanguinated by cardiac puncture and selected tissues and carcasses collected.

2. Analysis of Fecal and Urinary Samples: Excreta were collected at 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hour intervals. Excreta were freeze-trapped, weighed and stored at -15 °C until assayed. Cages were rinsed at the conclusion of the experiment. Fecal samples were homogenized in five volumes of distilled water and duplicate aliquots (1 ml) were combusted. Radioactivity was determined in duplicate aliquots of urine (100 µl) and cage wash (1 ml).

3. Analysis of Tissues and Carcass: Seven days post dosing, the animals were sacrifice and the following organs were collected: stomach, small intestine, large intestine, GI tract contents, brain, testes, ovaries, prostate, seminal vesicles, uterus, heart, kidneys, adrenal glands, thyroid glands, liver, lungs, blood, spleen, pancreas, and gross lesions. Weighed portion of muscle, fat and bone was also taken; The remaining bone, muscle and fat were processed with the carcass, which was ground to homogeneity. Tissues were weighed and stored at -15 °C until assayed. Small tissues, weighing less than 0.5 g, were combusted directly. Large tissues and ground carcass samples (5 g) were homogenized in 5 to 10 volumes of distilled water and duplicate 1 ml aliquots combusted.

C. Determination of Radioactivity and Data Analysis: Radioactivity of the combusted samples was measured using liquid scintillation counting for a maximum of 10 min or a statistical accuracy of ± 2%. Determination of background radiation was measured on fecal, urinary, tissue and carcass samples taken from naive control animals; samples were processed as outlined above. Sample radioactivity (minus background) was expressed as a percent of the total administered dose and ppm equivalents.

D. Statistics: Sample means and standard deviations were determined.

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IV. REGULATORY COMPLIANCE

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

V. RESULTS

A. Preliminary Experiments

1. Expired CO_2 Experiment: Preliminary experiments evaluated the excretion patterns of ^{14}C-ADBAC administered orally to male and female rats at a dose level of ≈10 mg/kg. Of the total dose administered the percent accountable as $^{14}CO_2$ ranged from 0.019 to 0.021%. Based on the results of this study, expired air was not monitored in the definitive experiments.

2. Preliminary Blood Level Experiment: The preliminary blood level experiment established that ^{14}C-ADBAC rapidly entered the systemic circulation. A peak radioactivity was detected three to four hours post dosing in males and three to eight hours in females. At 24 hours blood levels were decreased to 25%.

B. Definitive Experiments

1. Distribution of Radioactivity in Excreta: The urinary and fecal elimination for the different sampling intervals is in Table 1. For animals in the oral administration groups, peak urinary excretion occurred in the 12 to 24 hour interval. Those in the i.v. group showed peak urinary elimination within the first 4 hour of the study, with a minor secondary peak at 12 to 24 hours. For all study groups, fecal elimination peaked during the 12 to 24 hour interval.

The cumulative recovery of ^{14}C-labeled residues from urine and feces is summarized in Table 2. For oral administration, fecal elimination predominated and accounted for approximately 90% or more of the administered dose; urinary elimination accounted for less than 8%. The elimination pattern following i.v. administration showed higher percent of the dose appearing in the urine (31%, males; 21%, females); fecal elimination was correspondingly lower (44%, males; 55%, females).

2. Tissue Distribution of Radioactivity: The distribution of labeled residues in the tissues, carcass and blood was determined at terminal sacrifice

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(Tables 3 and 4). In general, accumulation of labeled residues in the various tissues was low (< 1%). Total accumulation tended to be slightly higher in males (0.16 to 0.58%) than females (0.03 to 0.15%). For i.v. administration, 33.4% (males) and 35.8% (females) of the total administered dose was associated with the carcasses, with high (but still less than 1%) appearing in the adrenals, heart, kidney, lung, muscle, prostate, seminal vesicles and thyroid.

As summarized in Table 5, essentially all (> 94%) of the administered radioactivity was accounted for in either the feces, urine, tissues or carcass.

VI. DISCUSSION AND COMMENTS

The absorption, distribution and excretion of ^{14}C-ADBAC was studied in male and female rats orally gavaged with low, high or repeated doses of test compound, additionally an i.v. low dose study was also performed. Following oral dosing, the greatest percentage of radioactive residues appeared in the feces (90 to 99%) with a lesser amount appearing in the urine (5.8 to 7.8%). The elimination pattern following i.v. administration differed markedly from the orally dosed animals. A much higher percentage of labeled residues appeared in the urine (20 to 30%); appreciable amounts still appeared in the feces (44 to 55%).

Tissue accumulation following oral administration also differed markedly from i.v. administered material. Whereas negligible amounts (< 1% of administered dose) accumulated in tissues for oral administration, accumulation was markedly higher (33.4% , males; 35.8%, females) for the i.v. route of administration.

Identification of metabolites were not performed in this study, However, the study indicates that such data will be included in a separate report.

CORE CLASSIFICATION: Supplementary. This study does not satisfy guideline requirements (§85-1) for a metabolism study in rats and is not acceptable for regulatory purposes. The study may be upgraded to guideline if metabolic profile data are submitted and judged to be acceptable.

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Table 1: Urinary and Fecal Elimination (% of total) (Data summarized from study Tables 54 and 55)

Time (hrs)	Low Dose ♂	Low Dose ♀	Repeat Low Dose ♂	Repeat Low Dose ♀	High Dose ♂	High Dose ♀	i.v. Low Dose ♂	i.v. Low Dose ♀
Urine								
0-4	0.70	1.29	0.90	1.26	0.79	1.67	10.23	7.74
4-8	1.18	0.84	0.61	0.49	2.03	2.13	1.86	2.60
8-12	1.60	0.38	0.47	0.18	0.60	0.48	3.96	1.23
12-24	1.56	3.54	1.54	1.33	0.86	0.97	4.08	2.00
24-36	0.32	0.14	0.35	0.62	0.38	0.32	2.15	1.27
36-48	0.11	0.40	0.49	1.02	1.62	0.70	1.48	0.91
48-72	0.14	0.17	0.29	0.[illegible]	0.42	0.34	2.26	1.39
72-96	0.04	0.04	0.03	0.13	0.30	0.09	1.55	1.32
96-120	0.03	0.03	0.02	0.09	0.39	0.08	1.15	0.78
120-144	0.02	0.02	0.01	0.10	0.22	0.06	0.90	0.77
144-168	0.03	0.01	0.01	0.06	0.08	0.05	0.90	0.48
Cage Rinse	0.02	0.03	0.02	0.04	0.05	0.05	0.11	0.09
Feces								
0-4	0.00	0.00	0.00	0.00	0.00	0.52	0.00	0.00
4-8	0.00	0.00	0.00	0.00	0.00	2.67	0.00	0.00
8-12	12.72	0.01	0.00	0.01	0.00	0.12	0.00	4.64
12-24	53.86	63.48	57.10	68.31	25.91	29.27	12.34	26.07
24-36	6.76	16.73	18.71	17.96	15.13	18.36	12.10	7.31
36-48	5.69	7.21	10.02	4.70	10.89	18.68	5.37	5.14
48-72	15.20	3.34	5.68	5.54	11.50	14.40	6.35	3.68
72-96	3.47	0.32	2.10	0.59	3.69	2.15	3.32	1.50
96-120	0.28	0.05	0.87	0.09	4.61	0.96	1.90	3.69
120-144	0.57	0.02	0.18	0.03	8.15	0.27	1.62	1.75
144-168	0.06	0.02	0.45	0.01	9.85	0.08	1.44	1.33

Low Dose: Single 10 mg/kg
High Dose: Single 50 mg/kg
Repeated Dose: 100 ppm dietary inclusion for 14 days, 10 mg/kg on Day 15
i.v. Dose: Single 10 mg/kg

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Table 2: Cumulative Percent Recovery of ^{14}C-Residues from Urine and Faces (Data summarized from study Tables 56 and 57)

Time (hrs)	Low Dose ♂	Low Dose ♀	Repeat Low Dose ♂	Repeat Low Dose ♀	High Dose ♂	High Dose ♀	I.v. Low Dose ♂	I.v. Low Dose ♀
Urine								
0-4	0.7	1.2	0.9	1.3	0.8	1.7	10.2	7.7
4-8	1.9	2.1	1.5	1.7	2.8	3.8	12.1	10.3
8-12	3.5	2.5	2.0	1.9	3.4	4.3	16.0	11.6
12-24	5.1	6.0	3.5	3.3	4.3	5.3	20.1	13.6
24-36	5.4	6.2	3.9	3.9	4.7	5.6	22.7	14.8
36-48	5.5	6.6	4.4	4.9	6.3	6.3	23.8	15.8
48-72	5.6	6.8	4.7	5.4	6.7	6.6	26.0	17.1
72-96	5.7	6.8	4.7	5.5	7.0	6.7	27.6	18.5
96-120	5.7	6.8	4.7	5.6	7.4	6.8	28.7	19.2
120-144	5.7	6.8	4.7	5.7	7.6	6.8	29.6	20.0
144-168	5.7	6.9	4.7	5.8	7.7	6.9	30.5	20.5
Cage Rinse	5.8	6.9	4.8	5.8	7.8	7.0	30.6	20.6
Faces								
0-4	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
4-8	0.0	0.0	0.0	0.0	0.0	3.2	0.0	0.0
8-12	12.7	0.0	0.0	0.0	0.0	3.3	0.0	0.0
12-24	66.6	63.5	57.1	68.3	26.2	32.6	12.3	30.7
24-36	73.3	80.2	75.8	86.3	41.3	50.9	24.4	38.0
36-48	79.0	87.4	85.8	92.0	52.2	69.6	29.8	43.2
48-72	94.2	90.8	91.5	96.5	63.7	84.0	36.2	46.8
72-96	97.7	91.1	93.6	97.1	67.4	86.2	39.5	48.3
96-120	98.0	91.2	94.5	97.2	72.0	87.1	41.4	52.0
120-144	98.6	91.2	94.7	97.2	80.2	87.4	43.0	53.8
144-168	98.6	91.2	94.7	97.2	90.0	87.5	44.4	55.1

Low Dose: Single 10 mg/kg
High Dose: Single 50 mg/kg
Repeated Dose: 100 ppm dietary inclusion for 14 days, 10 mg/kg on Day 15
i.v. Dose: Single 10 mg/kg

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Table 3: Tissue Distribution (ppm) of ^{14}C-labeled Residues. (Compiled from Tables of 30, 32, 34, 36, 38, 40, 42 and 44 of the study)

Tissue[a]	Low Dose		Repeat Low Dose		High Dose		i.v. Low Dose	
	♂	♀	♂	♀	♂	♀	♂	♀
Adrenals	0.08	0.07	0.07	0.12	0.66	0.49	8.18	6.27
Blood	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
Bone	0.00	0.00	0.01	0.00	0.00	0.00	1.15	0.36
Brain	0.00	0.00	0.01	0.01	0.00	0.00	0.12	0.11
Fat	0.00	0.01	0.00	0.01	0.00	0.00	0.05	0.05
GI Tract	0.00	0.00	0.01	0.01	0.30	0.01	0.64	0.55
GI Tract Cont.	0.00	0.00	0.05	0.00	0.44	0.02	0.13	0.06
Heart	0.05	0.03	0.05	0.07	0.29	0.24	21.04	17.52
Kidney	0.02	0.01	0.02	0.05	0.16	0.13	5.28	4.47
Liver	0.02	0.00	0.03	0.02	0.04	0.01	0.32	0.12
Lung	0.00	0.00	0.01	0.01	0.00	0.00	1.73	0.70
Muscle	0.01	0.00	0.00	0.01	0.01	0.02	2.72	2.65
Ovary	---	0.00	---	0.00	---	0.00	---	0.22
Pancreas	0.02	0.01	0.02	0.04	0.14	0.18	4.40	6.18
Plasma	0.01	0.01	0.01	0.01	0.03	0.05	0.02	0.02
Prostate	0.00	---	0.03	---	0.04	---	1.81	---
Sem. Vesicles	0.00	---	0.01	---	0.01	---	1.57	---
Spleen	0.00	0.00	0.01	0.01	0.01	0.01	0.63	0.37
Testes	0.00	---	0.00	---	0.00	---	0.08	---
Thyroid	0.00	0.00	0.02	0.01	0.07	0.05	4.44	5.21
Uterus	---	0.00	---	0.00	---	0.00	---	0.44
Carcass	0.02	0.00	0.00	0.01	0.01	0.06	3.44	3.53

[a] Weighed samples of bone, fat and muscle were taken at necropsy, remainder processed with the carcass.

Low Dose: Single 10 mg/kg
High Dose: Single 50 mg/kg
Repeated Dose: 100 ppm dietary inclusion for 14 days, 10 mg/kg on Day 15
i.v. Dose: Single 10 mg/kg

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Table 4: Tissue Distribution (% of total dose) of ^{14}C-labeled Residues. (Compiled from Tables of 31, 33, 35, 37, 39, 41, 43 and 45 of the study)

Tissue[a]	Low Dose		Repeat Low Dose		High Dose		I.V. Low Dose	
	♂	♀	♂	♀	♂	♀	♂	♀
Adrenals	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03
Blood	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bone	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01
Brain	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Fat	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GI Tract	0.00	0.00	0.00	0.00	0.02	0.00	0.33	0.46
GI Tract Cont.	0.01	0.00	0.28	0.01	0.46	0.03	0.74	0.63
Heart	0.00	0.00	0.00	0.00	0.00	0.00	0.80	0.74
Kidney	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.49
Liver	0.01	0.00	0.01	0.01	0.00	0.00	0.16	0.07
Lung	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.05
Muscle	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.22
Ovary	---	0.00	---	0.00	---	0.00	---	0.00
Pancreas	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.30
Plasma	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Prostate	0.00	---	0.00	---	0.00	---	0.05	---
Sem. Vesicles	0.00	---	0.00	---	0.00	---	0.03	---
Spleen	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01
Testes	0.00	---	0.00	---	0.00	---	0.01	---
Thyroid	0.00	0.00	0.01	0.00	0.00	0.00	0.03	0.03
Uterus	---	0.00	---	0.00	---	0.00	---	0.01
Carcass	0.15	0.02	0.02	0.05	0.09	0.11	30.2	32.7
Total	0.16	0.03	0.32	0.08	0.58	0.15	33.4	35.8

[a] Weighed samples of bone, fat and muscle were taken at necropsy, remainder processed with the carcass.

Low Dose: Single 10 mg/kg
High Dose: Single 50 mg/kg
Repeated Dose: 100 ppm dietary inclusion for 14 days, 10 mg/kg on Day 15
I.v. Dose: Single 10 mg/kg

Table 5: Total Percent Recovery of ^{14}C Residues in Urine, Feces, Carcass and Tissues (Data extracted from study Tables 46 to 53)

	Low Dose		Repeat Low Dose		High Dose		I.V. Low Dose	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine	5.8	6.9	4.8	5.8	7.8	7.0	30.6	20.6
Feces	98.6	91.2	95.2	97.2	90.2	87.5	44.4	55.1
Tissue and Carcass	0.2	0.0	0.3	0..	0.6	0.2	33.4	35.8
Total	104.5	98.1	100.2	103.1	98.4	94.6	108.4	111.5

Low Dose: Single 10 mg/kg
High Dose: Single 50 mg/kg
Repeated Dose: 100 ppm dietary inclusion for 14 days, 10 mg/kg on Day 15
I.v. Dose: Single 10 mg/kg

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Reviewed by: Robert F. Fricke, Ph.D. R. Fricke 23 Nov 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Jess Rowland, M.S. Jess Rowland 11/22/93
Section IV, Tox. Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: 90-Day Feeding - Rats (§82-1)

DP BARCODES: D188679, D189620 **SUBMISSION NO.:** S436173

P.C. CODE: 069105 **CASWELL NO.:** 016E

MRID NO.: 407466-01

TEST MATERIAL: ADBAC

SYNONYMS: Alkyl dimethyl benzyl ammonium chloride

STUDY NUMBER: 51-503

SPONSOR: ADBAC QUAT Joint Venture/Chemical Specialist Manufacturers Association, Washington, D.C.

TESTING FACILITY: Bushy Run Research Center, R.D. #4, Mellon Road, Export, PA

TITLE OF REPORT: Ninety-day dietary toxicity study with alkyl dimethyl benzyl ammonium chloride (ADBAC)

AUTHOR: J.P. Van Miller and E.V. Weaver

REPORT ISSUED: 20 June 1988

CONCLUSIONS: For 13 weeks, male and female rats were given diets containing 0, 100, 500, 1000, 4000, or 8000 ppm. The equivalent doses in mg/kg/day for the 100, 500, 1000, 4000 (estimated) and 8000 (estimated) ppm groups were 6.3, 31.2, 62.0, ≈ 248 and ≈ 496 for males and 0, 7.9, 38.3, 76.7, ≈ 308 and ≈ 616 for females. Treatment-related mortality was limited to the 4000 ppm (80% males and 73% females) and 8000 ppm (100% for males and females) groups. The 4000 ppm animals also showed decreased mean body weights, body weight gain and food consumption and increased incidence of gross and microscopic lesions.

	NOEL	LOEL
Males	500 ppm	1000 ppm
Females	1000 ppm	4000 ppm

The LOEL is based on decreased body weight and body weight gain in males and increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidence of gross and microscopic lesions in females.

CORE CLASSIFICATION: Guideline. This study satisfies guideline requirements (§82-1) for a 90-day feeding study in rats and is acceptable for regulatory purposes.

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A. MATERIALS

1. Test compound: ADBAC Description: viscous, pale-yellow colored liquid Batch #: 6158-59-60 Purity: 79.7% [a.i.] Contaminants: not given

2. Test animals: Species: Rat Strain: Sprague-Dawley CD Age: 7 weeks Weight (g): 221.6 - 250.9 (males), 150.1 - 174.4 (females) Source: Charles River Breeding Laboratories, Inc., Portage, MI. Housing: Individually in suspended cages Feed: Purina Certified Rodent Chow #5002 Water: Tap water, *ad libitum* Environment: Temperature, 66 to 75 °F; Humidity, 20 to 40%; Light cycle, 12 hr light/12 hr dark

B. METHODS:

1. Study Design: Male and female rats were exposed to test compound, at dietary concentrations of 0, 100, 500, 1000, 4000, or 8000 ppm, for 90 days. Animals were randomly assigned to study test groups as shown in Table 1.

Table 1: Animal Assignment to Study Groups

Study Group		Dose in Diet* (ppm)	Animals/Group Male	Female
Control	(CON)	0	15	15
Low	(LDT)	100	15	15
Middle 1	(MDT1)	500	15	15
Middle 2	(MDT2)	1000	15	15
Middle 3	(MDT3)	4000	15	15
High	(HDT)	8000	15	15

* Adjusted for percent purity of active ingredient

2. Diet preparation: A concentrated premix was prepared by thoroughly mixing test compound, adjusted for percent purity of active ingredient, with basal diet. The 8000 ppm diet was prepared by dilution of the premix with basal diet. The remaining diets were prepared by serial dilution of the next higher concentration diet with basal diet.

3. Statistical Evaluations: Parametric data were initially analyzed for homogeneity of variances using Levene's test. Homogeneous data were further analyzed using analysis of variance (ANOVA). Data sets yielding a significant ANOVA result were further analyzed using pooled variance t-tests. Heterogeneous data sets were analyzed using ANOVA for unequal variances followed by separate variance t-tests. Nonparametric data were analyzed using Kruskal-Wallis' test or the Wilcoxon rank sum test, as modified by Mann-Whitney. The Fisher's exact test was used to analyze frequency data.

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C. REGULATORY COMPLIANCE

1. Quality assurance was documented by signed and dated GLP and quality assurance statements.

2. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

3. A statement of "no confidentiality claims" was provided.

D. RESULTS:

1. Analysis of Test Diets: The prepared diets were analyzed for stability, homogeneity and concentration. Test diets were stable for at least 21 days at room temperature. Analysis of test diet samples, taken from the top, middle and bottom, indicated that the test compound was homogeneously distributed (coefficient of variation 2.6 to 5.0%) and within 98.3 and 113.9% of nominal concentrations. The dietary concentrations of test compound were also confirmed periodically throughout the study; concentrations were all within 95.2 to 111.8% of the nominal concentration.

2. Clinical Observations and Mortality: Animals were inspected once daily for signs of toxicity and twice daily for moribundity and mortality. A detailed clinical exam was performed once each week. Treatment-related mortality and moribund sacrifices were limited to the 4000 and 8000 ppm groups. At 4000 ppm, males experienced 80% lethality within the first 7 to 19 days of the study (mean time to death 10 days); for females 73% lethality occurred within 7 to 11 days (mean time to death = 8 days). At 8000 ppm, 100% lethality occurred within 6 to 8 days for males and 4 to 6 days for females; the mean times to death were 7 days and 5 days, respectively. Pertinent, treatment-related clinical observations were generally confined to the 4000 and 8000 ppm dose groups and consisted of emaciation, loose feces, red cutis, abdominal distension and perineal staining (Table 2).

3. Body Weight and Body Weight Gains: Animals were weighed at the start of the study, weekly during the study, and at terminal sacrifice.

a. Body weight (Appendix 1): Because of the high lethality at 8000 ppm, body weight data were only available for males at Week 1. At this time, the surviving animals showed a highly significant weight loss. At 4000 ppm, the body weights of the surviving males showed highly significant decreases in body weight throughout the entire study. At 1000 ppm, statistically significant decreases were noted only during Weeks 2 and 3. Females in the 4000 ppm group

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Table 2: Incidence of Clinical Observations (Data summarized from Appendix 5 and Tables 1 and 2 of the study)

Observation	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
Found dead	♂	0	0	0	0	10	15
	♀	0	0	0	0	5	15
Moribund sacrifice	♂	0	0	0	0	2	0
	♀	0	0	0	0	5	0
Loose Feces	♂	0	0	0	0	14	8
	♀	0	0	0	3	13	0
Red Cutis	♂	0	0	0	0	12	4
	♀	0	0	0	0	6	0
Emaciation	♂	0	0	0	0	11	6
	♀	0	0	0	1	9	0
Abdominal Distension	♀	0	0	0	0	5	0
Perineal staining	♂	0	0	0	0	2	5

showed highly significant decreases in body weights during the first four weeks of the study and biologically, but not statistically, significant decreases of 4.5 to 10.5% from Week 5 through 13.

b. Cumulative body weight gain (Appendix 2): The body weight gain data showed highly significant decreases for males at 8000 ppm (Week 1 only) and at 4000 ppm during the entire study. At 1000 ppm, significant decreases were observed for Week 0 to 1, 2, 3, 4, 5, and 6 and for Week 0 to 10. At other times, the body weight gains were lower than controls, but were comparable to the weight gains of animals in the 100 and 500 ppm groups. For females in the 4000 ppm group, a consistent, significant decrease in body weight gain was observed for most of the study. Females in the lower dose groups had body weigh gains comparable to controls.

4. Food Consumption and Achieved Compound Intake: Food consumption was measured at weekly intervals.

a. Food consumption: Food consumption was monitored at weekly intervals throughout the study. Because of the high mortality and excessive food spillage, food consumption data were available for 8000 ppm males for Week 1 and for 4000 ppm males and females intermittantly throughout the study . Significant decreases were observed in 8000 males and 4000 ppm males and females during Week 1 of the study (Table 3). For Weeks 2 and 3, males in the 1000 and 4000 ppm

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groups had significantly lower food consumption, while in females significant differences were noted only in the 4000 ppm group during Weeks 2, 9 and 10.

Table 3: Food Consumption (Data summarized from Tables 3 and 4 of study)

Sex	Week of Study	Food Consumption (g/animal/day) CON	LDT	MDT1	MDT2	MDT3	HDT
Male	1	24.9	24.6	24.1	24.2	6.7**	2.1**
	2	26.2	25.5	25.0	24.4*	16.4*	--
	3	27.4	26.3	26.0	25.3**	17.8**	--
Female	1	16.6	17.2	16.8	16.9	7.2**	--
	2	17.6	17.6	17.2	17.5	12.8**	--
	9	18.3	18.6	17.3	18.0	13.5**	--
	10	17.6	18.2	17.1	17.5	12.0**	--

* $p \leq 0.05$, ** $p \leq 0.01$

b. Achieved compound intake (Table 4): The actual compound intakes for animals in the 100, 500, and 1000 ppm groups are given in Table 4. Because of excessive mortality and food spillage, the values for animals in the 4000 and 8000 ppm were estimated by the reviewer.

Table 4: Compound Intake (Data summarized from Tables 5 and 6 of study)

Dose in Diet (ppm)	Compound Intake (mg/kg/day) Male	Female
100	6.3	7.9
500	31.2	38.3
1000	62.0	76.7
4000	≈ 248	≈ 308
8000	≈ 496	≈ 616

5. Ophthalmological Examinations: Examinations were performed on all animals immediately before terminal sacrifice; no prestudy examination was performed. At terminal sacrifice approximately 80% of the animals had corneal crystals; each study group had approximately the same incidence. In another subchronic feeding study (MRID No.: 409663-02) a prestudy examination revealed a high incidence of corneal crystals in all of the animals, indicating that the ocular lesions noted in the present study are not treatment-related. Other ocular findings were noted, but were sporadic in nature and not suggestive of treatment-related effects.

6. Clinical Pathology: At terminal sacrifice, clinical chemistry and hematological analyses were performed on blood

collected from the retroorbital sinus of fasted animals (10/sex/dose, if possible). The following hematology and serum chemistry parameters were evaluated:

Hematology Parameters

Hematocrit (HCT)	Reticulocyte count (RET)
Hemoglobin (HGB)	Leukocyte differential count (LDC)
Leukocyte count (WBC)	Mean corpuscular HGB (MCH)
Erythrocyte count (RBC)	Mean corpuscular HGB conc. (MCHC)
Platelet count (PLAT)	Mean corpuscular volume (MCV)

Serum Chemistry Parameters

Electrolytes
- Calcium (Ca)
- Chloride (Cl)
- Sodium (Na)
- Phosphorous (P_i)
- Potassium (K)

Enzymes
- γ-Glutamyl transpeptidase (GGT)
- Alkaline phosphatase (ALK)
- Aspartate aminotransferase (SGOT/AST)
- Alanine aminotransferase (SGPT/ALT)

Other
- Glucose (GLU)
- Blood creatinine (CREAT)
- Blood urea nitrogen (BUN)
- Phospholipid (PL)
- Total bilirubin (TBIL)
- Direct bilirubin (DBIL)
- Indirect bilirubin (IBIL)
- Protein, total (PROT)
- Triglycerides (TG)
- Albumin (ALB)
- Globulins (GLOB)
- A/G ratio (A/G)

Results: Significant changes in hematology and serum chemistry parameters are presented in Table 5, below. The hematology results for the treated males were comparable to control values. Females in the 4000 ppm group showed a statistically significant decrease in hemoglobin concentration. Males in the 4000 ppm group showed significantly elevated inorganic phosphate and alanine aminotransferase and significantly decreased serum glucose. The differences in the hematology and serum chemistry parameters appeared to be slight and of questionable biological significance due to lack of corroborative treatment-related histopathologic l lesions in the kidneys or liver.

Table 5: Serum Chemistry and Hematology Results (Data summarized from Appendix 3, Tables 2, 5 and 6)

Parameter	Sex	CON	LDT	MDT1	MDT2	MDT3	HDT
GLU (g/l)	♂	1.21	1.09**	1.09**	1.06***	0.95***	--•
ALT (U/l)	♂	14	15	16	14	21**	--
P_i (mg/l)	♂	59	59	60	61	80***	--
HGB (g/dl)	♀	15.5	15.8	15.5	15.7	14.9*	--

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$
• 100% mortality, no data available.

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7. Sacrifice and Pathology: Detailed pathological examination was performed on animals in the control and treatment groups. The tissues listed below were collected at necropsy and saved in 10% neutral buffered formalin. Histological examinations were performed on tissues (marked X or XX) from ten randomly selected animals of the control and 1000 ppm groups; for the remaining dose groups, the organs marked XX were examined from ten randomly selected animals in each dose group. Selected tissues (CAPITAL LETTERS) were weighed before being fixed.

Digestive system	Cardiovas./Hematol	Neurologic
X Pancreas	X Aorta	X BRAIN
X Salivary glands	X Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
XX Stomach	X Lymph nodes	X Pituitary
XX Duodenum	X Spleen	Eyes
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	X ADRENALS
X Cecum	XX KIDNEYS	Lacrimal gland
X Colon	X Urinary bladder	Mammary gland
X Rectum	XX TESTES	X Parathyroids
XX LIVER	X Epididymides	X Thyroids
Respiratory	X Prostate	Other
X Trachea	Cervix	XX Gross lesions
XX Lungs	Vagina	Skin
	X OVARIES	X Skeletal muscle
	X Uterus	Bone

a. Organ weights: At scheduled sacrifice, the terminal body weights and absolute and relative (% of body weight and % of brain weight) organ weights were determined, Table 6. Terminal body weights were significantly decreased in the 4000 ppm males and females. Expressed as a percent of body weight, the relative brain (males and females), liver (females) and testicular weights were significantly increased in the 4000 ppm animals. Expressed as a percent of brain weight, the relative weights of liver, kidney, heart for the 4000 ppm males were significantly higher than controls. Changes in organ weights were attributed to lowed terminal body weights rather than to treatment.

b. Gross pathology: Gross pathological examinations were performed on animals found dead or sacrificed in moribund condition (tissue integrity permitting) during the study and surviving animals at terminal sacrifice. Treatment-related gross lesions are summarized in Table 7, below. A high number of animals in the 4000 and 8000 ppm groups were emaciated and thin at the time of the examination. From the stomach to the cecum, the intestines were distended and fluid filled. The jejunum and ileum had abnormal contents, while a color change was evident in the stomach. Color changes were

Table 6: Terminal Body Weights and Absolute and Relative Organ Weights (Data summarized from Tables 11, 12, 13, 14 and 15 of study)

Observation	Sex	CON	LDT	HDT1	HDT2	HDT3	HDT
Terminal Body Weights (g)							
	♂	509	490	492	491	341**	—[a]
	♀	257	260	260	257	211**	—
Absolute Organ Weights (g)							
Liver	♂	13.3	12.3	13.1	12.7	9.12**	—
Kidney	♂	3.32	3.28	3.31	3.33	2.35**	—
Spleen	♂	0.70	0.66	0.66	0.70	0.52**	—
Heart	♂	1.45	1.41	1.42	1.42	1.13**	—
Organ Weights Relative to Terminal Body Weight (%)							
Brain	♂	0.413	0.435	0.425	0.428	0.587**	—
	♀	0.740	0.725	0.719	0.725	0.885**	—
Liver	♀	2.57	2.52	2.53	2.56	3.15**	—
Testes	♂	0.687	0.697	0.711	0.720	0.982**	—
Organ Weights Relative to Brain Weight (%)							
Liver	♂	634	581	629	609	456**	—
Kidneys	♂	159	155	159	159	117**	—
Heart	♂	69.5	66.4	68.4	67.7	56.3**	—

** $p < 0.01$

a 100% mortality, no data available.

also apparent in the lungs (4000 and 8000 ppm males and females) and kidneys (8000 ppm males only). In the 4000 and 8000 ppm groups, brain hemorrhage (males and females), fluid filled urinary bladders (males only) and spleens with decreased size (males only).

c. Microscopic pathology: Significant, treatment-related, microscopic lesions were observed only in the 4000 and 8000 ppm animals (Table 8). Males in the 4000 and 8000 ppm groups showed significantly higher incidence of intestinal lesions, which included congestion and edema in stomach, congestion in the jejunum and mucosal cell necrosis in the cecum. The lungs and brains of 4000 and 8000 ppm males and females were hemorrhaged and/or congested. Other significant lesions included contracted spleens (4000 ppm males), histiocytic aggregates and/or mastocytosis of the mesenteric lymph nodes (4000 ppm, both sexes) and congestion and/or hepatocellular atrophy in the liver (4000 and 8000 ppm, both sexes). Other lesions were observed, however, their occurrences were not dose-related and appeared to be sporadic in nature.

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Table 7: Incidence of Gross Pathological Observations - All Deaths Combined (Data summarized from Appendix 2, Tables 1 to 4 of the study)

Observation	Sex	CON	LDT	MDT1	MDT2	HDT3	HDT
BODY - Emaciated/Thin	♂	0	0	0	0	12	14
	♀	0	1	0	0	12	13
STOMACH - Color change	♂	0	0	1	0	4	8
	♀	0	0	0	0	3	4
- Fluid	♂	0	0	0	0	3	11
	♀	0	0	0	0	1	6
JEJUNUM - Contents abnormal	♂	0	0	0	0	7	14
	♀	0	0	0	0	5	7
- Fluid	♂	0	0	0	0	2	1
	♀	0	0	0	0	5	1
ILEUM - Contents abnormal	♂	0	0	0	0	7	14
	♀	0	0	0	0	5	7
- Fluid	♂	0	0	0	2	1	14
	♀	0	0	0	0	5	2
CECUM - Fluid	♂	0	0	0	0	11	13
	♀	0	0	0	0	11	12
SPLEEN - Decreased size	♂	0	0	0	0	5	1
LUNGS - Color change	♂	0	0	0	0	6	9
	♀	0	0	0	0	1	10
KIDNEYS - Color change	♂	0	0	0	0	0	5
BRAIN - Hemorrhage	♂	0	1	0	0	2	3
	♀	0	0	0	0	4	8
URINARY BLADDER - Fluid	♂	0	0	0	0	2	9

. Combined deaths includes all animals found dead, sacrificed in moribund condition sacrifice by design.

D. DISCUSSION: For 13 weeks, male and female rats were given diets containing 0, 100, 500, 1000, 4000, or 8000 ppm. The equivalent doses in mg/kg/day for the 100, 500, 1000, 4000 and 8000 ppm groups were 6.3, 31.2, 62.0, ≈ 248, and for males and 0, 7.9, 38.3, 76.7, ≈ 308 and ≈ 616 for females, respectively. ((Because of high mortality and food spillage, compound intakes for the 4000 and 8000 ppm groups were estimated by reviewer.) During the study, toxic responses included a higher incidence of clinical signs, decreased food consumption and decreased mean body weight and body weight gains. Treatment-related clinical signs consisted of loose feces, red cutis, and emaciation in both males and females, abdominal distention in females and perineal staining in males. These observations were noted most frequently in the 4000 ppm group, and to a lesser extent at 8000 ppm. Food consumption during the study were incomplete for the 4000 and 8000 ppm animals due to high mortality and food spillage. The data which are available indicate a marked reduction in food

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Table 8: Incidence of Microscopic Observations - All Deaths Combined (Data summarized from Appendix 2, Tables 5 and 6 of the study)

Observation	Sex	COM	LOT	MDT1	MDT2	MDT3	HDT
STOMACH							
- Congestion	♂	0/10	0/10	0/10	0/10	4/9	6/9**
- Edema	♂	0/10	0/10	0/10	0/10	6/9**	6/9**
JEJUNUM							
- Congestion	♂	0/10	0/0	0/0	0/10	1/4	2/2*
CECUM							
- Mucosal cell necrosis	♂	0/10	0/0	0/0	0/10	1/4	4/5**
SPLEEN							
- Contracted	♂	0/10	0/0	0/0	0/10	4/4**	1/1
MESENTERIC LYMPH NODE							
- Histiocytic aggregates	♂	0/10	0/0	0/0	0/0	3/3**	0/0
- Mastocytosis	♀	0/10	0/0	0/1	0/10	3/3**	0/0
BRAIN							
- Congestion	♂	0/10	0/0	0/0	0/0	1/1	2/2*
	♀	0/10	0/0	0/0	0/10	1/1	7/7*
LUNGS							
- Congestion	♂	0/10	0/10	0/9	0/10	5/10*	8/10**
	♀	0/10	0/10	0/10	1/10	4/10	9/10**
- Hemorrhage	♂	1/10	1/10	1/9	1/10	2/10	7/10*
	♀	0/10	0/10	1/10	1/10	2/10	6/10*
LIVER							
- Congestion	♂	0/10	0/10	0/10	0/10	7/10**	10/10**
	♀	0/10	0/10	0/10	0/10	4/10	10/10**
- Hepatocellular atrophy	♂	0/10	0/10	0/10	0/10	7/10**	8/10**
	♀	0/10	0/10	0/10	0/10	4/10	7/10**

* $p \leq 0.05$, ** $p \leq 0.01$

consumption by 8000 ppm males during Week 1 and 4000 ppm males through Week 3 and 4000 ppm females through Week 2 and Weeks 9 and 10 of the study. The 1000 ppm males also showed a slight, but significant, decrease during Weeks 2 and 3. Since, at other times during the study, food consumption by these animals was comparable to control values, the observed decreases may be a reflection of food palatability, rather than an effect of treatment.

Clinical pathology results did not reveal any biologically meaningful differences attributable to treatment. Although statistically significant differences were observed in serum glucose, alanine aminotransferase, inorganic phosphate and hemoglobin, the magnitude of the changes was considered slight and within the range of published historical control data.

At terminal sacrifice, significant decreases were observed in the mean body weights of 4000 ppm males and females. The significant differences in the absolute and relative organ weights appear to

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be a reflection of the significantly lower terminal body weights of the 4000 ppm animals, rather than a treatment-related effect.

Gross and histopathological examination revealed significant treatment-related lesions, particularly in the intestinal tract. Animals in the 4000 and 8000 ppm groups had a high incidence of fluid filled intestinal tracts (stomach to cecum). The jejunum and ileum had abnormal contents, while a color change was evident in the stomach. Microscopic evaluation revealed edema and/or congestion of the stomach and jejunum. Mucosal cell necrosis was evident in the cecum. Other significant lesions included contracted spleens (4000 ppm males), histiocytic aggregates and/or mastocytosis of the mesenteric lymph nodes (4000 ppm, both sexes) and congestion and/or hepatocellular atrophy in the liver (4000 and 8000 ppm, both sexes). Other lesions were observed, however, their occurrences were not dose-related and appeared to be sporadic in nature.

From the results of this study, NOEL and LOEL are as follows:

	NOEL	LOEL
Males	500 ppm (31.2 mg/kg/day)	1000 ppm (62.0 mg/kg/day)
Females	1000 ppm (76.7 mg/kg/day)	4000 ppm (≈ 308 mg/kg/day)

The LOEL is based on decreased body weight and body weight gain in males and increased mortality, decreased mean body weights, body weight gain and food consumption, and increased incidence of gross and microscopic lesions.

Core Classification: Guideline. This study satisfies guideline requirements (§82-1) for a 90-day feeding study in rats and is acceptable for regulatory purposes.

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