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Eastman Chemical Company
P. O. Box 511
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Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington, DC 20460-0001
ATTN: 8(d) Health and Safety Reporting Rule (Notification/Reporting)

CONTAIN NO CBI

Dear Administrator,

The Eastman Chemical Company has reviewed their files in response to requests by the Environmental Protection Agency to report unpublished health and safety data pursuant to Section 8(d) of the Toxic Substances Control Act (71 Fed. Reg. 47,130, August 16, 2006; 71 Fed. Reg. 57,439, September 29, 2006).

The table below lists those chemicals for which Eastman possessed health and safety data. Copies of this data are organized by the chemical's CAS number on the enclosed compact disc. Please contact Dr. James A. Deyo at 423-229-5208 should you have any problems extracting the information from the disc.

CAS No.	Chemical Name
110-18-9	1,2-Ethanediamine, N,N,N',N'-tetramethyl-
110-33-8	Hexanedioic acid, dihexyl ester
111-85-3	Octane, 1-chloro-
124-63-0	Methanesulfonyl chloride
131-57-7	Methanone, (2-hydroxy-4-methoxyphenyl)phenyl-
25646-71-3	Methanesulfonamide, N-[2-[(4-amino-3-methylphenyl)ethylamino]ethyl]-, sulfate (2:3)
4170-30-3	2-Butenal
645-62-5	2-Hexenal, 2-ethyl-
68987-66-6	Ethene, hydrated, by-products from
7795-95-1	1-Octanesulfonyl chloride
81-07-2	1,2-Benzisothiazol-3(2H)-one, 1,1-dioxide
94-96-2	1,3-Hexanediol, 2-ethyl-
97-00-7	Benzene, 1-chloro-2,4-dinitro-

Regards,

Marc G. Schurger
Director, Product Safety & Health

8 6 0 7 0 0 0 0 0 1

Enclosure: Compact Disc labeled "Eastman TSCA 8(d) Submission; October 4, 2006

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Eastman Attachments (MR-29312)

81-07-2

94-96-2

97-00-7

110-18-9

110-33-8

111-85-3

124-63-0

131-57-7

4170-30-3

7795-95-1

25646-71-3

68987-66-6

FINAL REPORT

COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 **EAN: 905401**
CAS No.: 025646-71-3 **HAEL No.: 97-0220**

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

GUIDELINE

OECD: 404
EEC: Annex V., Test B.4

AUTHOR

Kenneth P. Shepard, B.S.

TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

LABORATORY PROJECT ID

97-0220A2

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

STUDY COMPLETION DATE

October 21, 1997

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 97-0220-1 STUDY DIRECTOR: SHEPARD,K.P.
ACCESSION NUMBER: 905401

PAGE 1
10/17/97

STUDY TYPE: ACUTE DERMAL IRRITATION TEST

M L James
(AUDITOR, QUALITY ASSURANCE UNIT)

10/17/97
DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
10/07/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
10/09/97	CLINICAL SIGNS AT 48 HRS.	10/17/97
10/17/97	FINAL REPORT REVIEW	10/17/97

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

Annex 2, Organisation for Economic Cooperation and Development, Guidelines for Testing of Chemicals [C(81)30(Final)].

Kenneth P. Shepard, B.S.
Study Director

October 21, 1997
Month/Day/Year

SIGNATURE PAGE

Kenneth P. Shepard, B.S.
Study Director

October 21, 1997
Month/Day/Year

Douglas C. Topping, Ph.D.
Unit Director, Mammalian Toxicology

Oct 20, 1997
Month/Day/Year

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ABSTRACT

COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 **EAN: 905401**
CAS No.: 025646-71-3 **HAEL No.: 97-0220**

ACUTE DERMAL IRRITATION STUDY IN THE RABBIT

A dermal irritation study was conducted by administering single topical doses of 0.5 gram of the test substance to rabbits. The test substance was left in contact with the skin under an occlusive wrap for four hours. Skin lesions were graded according to OECD Guideline 404 (Annex V., Test B.4).

No irritant response or serious lesion was noted during the 72-hour observation period. Based on these results, the test substance requires no skin irritation classification as defined in the 18th Adaptation of the EC Classification, Packaging, and Labelling of Dangerous Substances Directive.

STUDY AND TEST SUBSTANCE INFORMATION

Testing Facility

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272

Project Participants

Study Director:	Kenneth P. Shepard, B.S.
Principal Investigator:	John W. Mosher, B.S.
Veterinarian:	Milan S. Vlaovic, D.V.M., Ph.D.
Report Author:	Kenneth P. Shepard, B.S.

Sponsor

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

Sponsor's Representative:
Karen R. Miller, Ph.D.

Test Substance Characterization

Chemical Identity:	Color Developing Agent CD-3
Chemical Name:	N-(2-((4-Amino-3-methylphenyl)ethylamino) ethyl)methanesulfonamide sulfate (2:3) monohydrate
CAS No.:	025646-71-3
PM No.:	01578-00
EAN:	905401
HAEL No.:	97-0220
SRID or Lot No.:	75168
Physical State and Appearance:	Solid, Off-white powder
Source of Test Substance:	Eastman Chemical Company
Laboratory Project ID:	97-0220A2

Study Dates

Study Initiation Date:	October 7, 1997
Experimental Start Date:	October 7, 1997
Experimental Completion Date:	October 10, 1997

PURPOSE

The purpose of the study was to determine the potential of the test substance to cause primary irritation of mammalian skin.

MATERIALS AND METHODS

Test system

Three albino rabbits (Hra:(NZW)SPF) obtained from Covance Research Products Inc. (Denver, PA) were assigned to the study. The rabbits were young adults (at least three months old) and weighed at least 2000 grams at the start of the study. Rabbits were chosen for this study because they are a common representative species for dermal irritation studies. The rabbit is the preferred species recommended for use in the OECD Guideline.

Husbandry

Housing

Animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited vivarium in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The rabbits were singly housed in suspended, stainless-steel mesh cages. Cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed every other day.

Environmental Conditions

The study room was maintained at 19-21°C and 60-68% relative humidity. A photoperiod of 12 hours light from 6 a.m. to 6 p.m. was maintained.

Acclimation Period

The animals were isolated upon arrival and allowed to acclimate for a period of 5 days. Animals were judged to be healthy prior to testing.

Feed

Certified High Fiber Rabbit Diet (PMI® #5325) was available *ad libitum*. Feed containers were cleaned weekly and refilled at least once a week. No known contaminants which would interfere with the outcome of this study were present in the feed. Analyses of feed are maintained on file within the testing laboratory.

Husbandry, continued

Water

Water was available *ad libitum* through an automatic watering system. The source of the water was the local public water system. There have been no contaminants identified in previous water analyses that would be expected to interfere with the conduct of the study. Semiannual analyses of water are maintained on file within the testing laboratory.

Identification

Upon arrival, all rabbits were identified by uniquely-numbered ear tags. Cage cards contained the study-specific animal number and the ear tag number.

Experimental Design

Test Procedures

This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals: Guideline 404, Acute Dermal Irritation/Corrosion; and European Economic Community (EEC): Annex V., Test B.4, Acute Toxicity (Skin Irritation).

Identification Numbers of Animals Used

Animal numbers 340, 341, and 342 were used in this study.

Preparation of Test Substance

The test substance was administered as a solid moistened thoroughly with water.

Test Substance Exposure

The hair was removed from an area of the dorsal skin with an electric clipper. A single dose of 0.5 gram of the test substance was applied topically to each animal using a fiber pad. An occlusive wrap was used to hold the pad with the test substance in place for a period of four hours. At the end of the exposure period, the application site was rinsed with running water.

Vehicle

No vehicle was used.

Experimental Design, continued

Control Substance

No control substance was used. Adjacent areas of untreated skin of each animal served as control sites for the test areas.

Clinical Observations

The site of application was examined at 1, 24, 48, and 72 hours after removal of the occlusive patch. Observations included estimation of erythema, edema, necrosis, eschar formation, scarring, erosion, and staining caused by the test substance as well as general systemic effects.

Grading the Irritant Response

The most severely affected area within the sites of application of the test substance were examined and grades of dermal reactions recorded for each animal at all observation periods. Skin reactions were graded and scored as described in Table 1.

TABLE 1 (Grading Of Skin Reaction[1])

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm and extending beyond area of exposure)	4

[1] Graded as described in OECD Guideline 404 (Annex V., Test B.4) (Grading of Skin Reaction)

Grading Other Clinical Observations

Other serious skin lesions, signs of abnormality, or toxic effects were graded and scored as described in Table 2.

Experimental Design, continued

Grading Other Clinical Observations, continued

TABLE 2 (Grading Of Other Clinical Observations)

Degree of Severity	
Very Slight	1
Slight	2
Moderate	3
Severe	4

Body Weight

Body weights were measured on the day of initiation of the study.

Necropsy

No necropsies were conducted at the conclusion of the 72-hour observation period.

Data Storage

The final report, data sheets, all nonperishable raw data, and an aliquot of the test substance have been stored in the testing facility archive managed under GLP-mandated conditions.

Protocol and Standard Operating Procedure Deviations

The were no SOP or protocol deviations during the study.

RESULTS

Observations for Skin Irritation

The application site of each animal was examined for signs of irritation at 1, 24, 48, and 72 hours after termination of exposure to the test substance. Observations for irritation (erythema, edema) are listed in Table 3.

TABLE 3 (Observations For Skin Irritation)

ANIMAL NUMBER	Erythema, Edema[1]			
	1 HOUR	24 HOURS	48 HOURS	72 HOURS
340	0,0	0,0	0,0	0,0
341	0,0	0,0	0,0	0,0
342	0,0	0,0	0,0	0,0

[1] Graded as described in OECD Guideline 404 (Annex V., Test B.4) (Grading of Skin Reaction)

Description of Serious Lesions and Irritation Other Than Erythema and Edema

No irritant response or serious lesion was noted during the 72-hour observation period.

Toxic Effects

No toxic effects were noted during the study.

Body Weights

At initiation of the study, Rabbit Numbers 340, 341, and 342 weighed 3827, 3473, and 3913 grams, respectively.

DISCUSSION

Since no signs of irritation were evident at any time during the study, the test substance was not considered a dermal irritant.

CONCLUSION

Based on the lack of an irritant response, the test substance requires no skin irritation classification as defined in the 18th Adaptation of the EC Classification, Packaging, and Labelling of Dangerous Substances Directive.

REFERENCES

National Research Council (1996). *Guide for the Care and Use of Laboratory Animals.* National Academy Press. Washington, D.C.

FINAL REPORT

"EASTMAN" COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 EAN: 905401
CAS No.: 025646-71-3

ACUTE DERMAL TOXICITY AND IRRITATION STUDY IN THE GUINEA PIG

AUTHOR

Kenneth P. Shepard, B.S.

TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

REPORT DATE

August 7, 1997

"EASTMAN" COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 EAN: 905401
CAS No.: 025646-71-3

ACUTE DERMAL TOXICITY AND IRRITATION STUDY IN THE GUINEA PIG

A dermal toxicity and irritation study was conducted in guinea pigs using a standardized method developed by the Laboratory of Industrial Medicine, Eastman Kodak Company. The study was initiated on October 12, 1949. All data from this study were stored in the testing facility archive managed under GLP-mandated conditions. This report was prepared from the archived raw data.

For this study, three guinea pigs were used. Prior to the day of exposure to the test substance (Day 0), individual body weights were recorded and the backs of the guinea pigs were clipped and depilated. Each guinea received a volume of 20 mL/kg of either a 0.1, 0.5, or 1.0% solution of the test substance prepared in 2% sodium carbonate. The dose was administered using a gauze pad applied to the backs of the guinea pigs under a rubber cuff for a period of 24 hours. After termination of exposure (Day 1), observations for irritation (redness and edema) at the site of application and any clinical signs of toxicity were made. Observations were again performed on Day 9 and at termination of the study (Day 16).

The dose levels, animal numbers, individual body weights, percent of test substance in the dose preparations, and total volumes of test preparation administered are listed in Table 1. Observations for irritation and toxicity evident during the study are presented in Table 2.

No mortality and no signs of toxicity were noted in guinea pigs administer a topical dose of 200 mg/kg (1% solution) for an exposure period of 24 hours. Signs of irritation noted after this exposure period were limited to slight redness and edema at a dose level of 20 mg/kg and slight redness with moderate edema at dose levels of 100 and 200 mg/kg.

TABLE 1

Dose Level (mg/kg)	Animal Number	Body Weight (g)	Test Preparation (% Test Substance)	Dose Volume (mL/kg)	Actual Volume Administered (mL)
20	219R	602	0.1	20	12.04
100	220R	618	0.5	20	12.36
200	221R	634	1.0	20	12.68

TABLE 2

Dose Levels (mg/kg)	Animal Numbers	Observations for Irritation and Toxicity		
		Day 1 (10/12/49)	Day 9 (10/21/49)	Day 16 (10/28/49)
20	219R	Slight redness with slight edema No signs of toxicity	No signs of irritation No signs of toxicity	No signs of irritation No signs of toxicity
100	220R	Slight redness with moderate edema No signs of toxicity	No signs of irritation No signs of toxicity	No signs of irritation No signs of toxicity
200	221R	Slight redness with moderate edema No signs of toxicity	No signs of irritation No signs of toxicity	No signs of irritation No signs of toxicity

Slight staining at the application site was also noted for two guinea pigs on Days 1 and 9.

DISCUSSION

No signs of toxicity were noted for this test substance when administered topically to guinea pigs at a dose level of 200 mg/kg for a period of 24 hours. Signs of irritation caused by the test substance after this 24 hour exposure were limited to slight redness and moderate edema at the highest dose level of 200 mg/kg. Eight days after termination of exposure, no signs of irritation were evident.

CONCLUSION

The LD_{50} for this test substance when administered topically to guinea pigs for a period of 24 hours was greater than 200 mg/kg. Based on the conditions and results of this study, the test substance appears to be, at most, a slight skin irritant.

FINAL REPORT

COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 **EAN: 905401**
CAS No.: 025646-71-3 **HAEL No.: 97-0220**

ACUTE DERMAL TOXICITY STUDY IN THE RAT

GUIDELINE

OECD: 402
EEC: Annex V., Test B.3

AUTHOR

Kenneth P. Shepard, B.S.

TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

LABORATORY PROJECT ID

97-0220A1

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

STUDY COMPLETION DATE

November 26, 1997

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 97-0220-1 STUDY DIRECTOR: SHEPARD,K.P. PAGE 1
ACCESSION NUMBER: 905401 11/17/97

STUDY TYPE: ACUTE DERMAL TOXICITY

M. L. James
(AUDITOR, QUALITY ASSURANCE UNIT)

11/17/97
DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
10/07/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
10/09/97	CLINICAL SIGNS AT 48 HRS.	11/17/97
11/11/97	GROSS PATHOLOGY PATHOLOGY REPORT	11/11/97
11/17/97	FINAL REPORT REVIEW	11/17/97

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

Annex 2, Organisation for Economic Cooperation and Development, Guidelines for Testing of Chemicals [C(81)30(Final)].

Kenneth P. Shepard, B.S.
Study Director

November 26, 1997
Month/Day/Year

SIGNATURE PAGE

Kenneth P. Shepard, B.S.
Study Director

November 26, 1997
Month/Day/Year

Douglas C. Topping, Ph.D.
Unit Director, Mammalian Toxicology

Nov 20, 1997
Month/Day/Year

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ABSTRACT

COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 **EAN: 905401**
CAS No.: 025646-71-3 **HAEL No.: 97-0220**

ACUTE DERMAL TOXICITY STUDY IN THE RAT

An acute dermal toxicity study was conducted with a group of five male and five female rats administered a single limit dose of 2000 mg/kg of the test substance topically. The test substance was administered as a solid moistened thoroughly with water.

No mortality was noted during the study. Discoloration (black) of the hair immediately adjacent to the application site by the test substance was noted for all animals. The discolored hair was initially seen at termination of exposure and persisted to the end of the 14-day observation period. No abnormal clinical signs were noted at the site of application of the test substance, and no signs of toxicity were observed during the study. All animals gained weight during both weeks of the study. No treatment-related changes were observed at necropsy, and no tissues were collected for histological examination.

The acute dermal LD_{50} for this test substance was greater than 2000 mg/kg for male and female rats. The test substance was classified as, at most, slightly toxic according to the criteria set forth by Hodge and Sterner (1949) and requires no toxicity classification as defined in the 18th Adaptation of the EC Classification, Packaging, and Labelling of Dangerous Substances Directive.

STUDY AND TEST SUBSTANCE INFORMATION

Testing Facility

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

Project Participants

Study Director:	Kenneth P. Shepard, B.S.
Principal Investigator:	John W. Mosher, B.S.
Pathologist/Veterinarian:	Milan S. Vlaovic, D.V.M., Ph.D.
Report Author:	Kenneth P. Shepard, B.S.

Sponsor

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

Sponsor's Representative:
Karen R. Miller, Ph.D.

Test Substance Characterization

Chemical Identity:	Color Developing Agent CD-3
Chemical Name:	N-(2-((4-Amino-3-methylphenyl)ethylamino) ethyl)methanesulfonamide sulfate (2:3) monohydrate
CAS No.:	025646-71-3
PM No.:	01578-00
EAN:	905401
HAEL No.:	97-0220
SRID or Lot No.:	75168
Physical State and Appearance:	Solid, Off-white powder
Source of Test Substance:	Eastman Chemical Company
Laboratory Project ID:	97-0220A1

Study Dates

Study Initiation Date	October 7, 1997
Experimental Start Date	October 7, 1997
Experimental Completion Date	November 12, 1997

PURPOSE

The purpose of the study was to determine the estimated dermal LD_{50} of the test substance in male and female rats and the clinical signs of toxicity associated with a single topical dose.

MATERIALS AND METHODS

Test system

Five male and five female Sprague-Dawley® rats [SAS:VAF®(SD)] obtained from SASCO, Inc., Stone Ridge (Kingston), NY were randomly assigned to each dose group. At study initiation, male rats were 8 weeks of age and weighed 206 to 220 grams and female rats were 10 weeks of age and weighed 210 to 214 grams. Rats were chosen for this study because they are a common representative species for toxicity studies. The rat is one of the two primary rodent species recommended for use in the OECD Guideline.

Husbandry

Housing

Animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited vivarium in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The rats were singly housed in suspended, stainless-steel, wire mesh cages. Cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed at least three times a week.

Environmental Conditions

The study room was maintained at 19-23° C and 48-62% relative humidity. A photoperiod of 12 hours light from 6 a.m. to 6 p.m. was maintained.

Acclimation Period

The animals were isolated upon arrival and allowed to acclimate for a period of 5 days. Animals were judged to be healthy prior to testing.

Husbandry, continued

Feed

Certified Rodent Diet (Purina Rodent Chow #5002, pellets) was available *ad libitum*. Feed containers were cleaned and refilled at least once a week. No known contaminants which would interfere with the outcome of this study were present in the feed. Analyses of feed are maintained on file within the testing laboratory.

Water

Water was available *ad libitum* through an automatic watering system. The source of the water was the local public water system. There have been no contaminants identified in periodic water analyses that would be expected to interfere with the conduct of the study. Semiannual analyses of water are maintained on file within the testing laboratory.

Identification

Upon arrival, all rats were identified by uniquely-numbered metal ear tags. During randomization, study-specific animal numbers were assigned to each animal. Cage cards contained the study-specific animal number and the ear tag number.

Experimental Design

Test Procedures

This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals Guideline: 402, Acute Dermal Toxicity; and European Economic Community (EEC): Annex V., Test B.3 , Acute Toxicity (Dermal).

Randomization

The procedure for including animals in the study was to randomly select and assign animals from the same shipment to the study. Randomization was done by computer-generated lists. After assignment of animals to the study, the body weights were determined to ensure that variation in individual body weights did not exceed 20% of the mean weight for each sex.

Experimental Design, continued

Determination of Dose Levels

A limit dose of 2000 mg of the test substance/kg body weight was selected as the dose level for the dermal toxicity study.

Preparation of Test Substance

The test substance was administered as a solid moistened thoroughly with water.

Test Substance Exposure

The hair was removed from an area of the dorsal skin with an electric clipper. A single dose of the test substance was placed in contact with the skin using a fiber pad and an occlusive wrap to hold the test material in place for 24 hours. At the end of the exposure period, any residual test material was removed with running water.

Distribution of Animals

TABLE 1

Dose Level	Number of Animals	Animal Numbers	
		Males	Females
2000 mg/kg	5 Males & 5 Females	661 - 665	666 - 670

Body Weights

Body weights were measured on Days 0 (prior to treatment), 7, and 14.

Clinical Observations

Animals were observed at least once during the exposure period, and once each day thereafter for the duration of the experiment. Observations included, but were not limited to, examination of the hair, skin, eyes, mucous membranes, motor activity, feces, urine, respiratory system, circulatory system, autonomic nervous system, central nervous system, and behavior patterns.

Necropsy

All animals were euthanatized and necropsied at the completion of the 14-day observation period.

Data Storage

The final report, data sheets, all nonperishable raw data, and an aliquot of the test substance have been stored in the testing facility archive managed under GLP-mandated conditions.

Statistical Procedures

No statistical procedures were required during the study. No dose/mortality curve was constructed since graphs become statistically useful only with the use of large numbers of animals and dose groups.

Protocol and Standard Operating Procedure Deviations

The were no SOP or protocol deviations during the study.

RESULTS

Mortality

The number of animals dosed and the number of deaths at each dose level are listed in Table 2.

TABLE 2

Mortality Table

Dose (mg/kg)	Number of Rats Exposed (Male, Female)	Number of Deaths (Male, Female)	Time of Death
2000	5,5	0,0	Not Applicable

LD_{50} for male rats: > 2000 mg/kg (95% C.I. = No Range Calculable)
LD_{50} for female rats: > 2000 mg/kg (95% C.I. = No Range Calculable)

Clinical Signs

The time of each observation and the number of animals involved at each dose level are listed in Table 3.

TABLE 3

Table Of Clinical Observations

Dose (mg/kg)	Time	Clinical Signs	Number of Animals Affected
2000	Day 0 (During Exposure)	Appeared Clinically Normal	5/5 Males, 5/5 Females
2000	Day 1 to Day 14	Discoloration (Black) of the Hair Immediately Adjacent to the Application Site by the Test Substance	5/5 Males, 5/5 Females

Body Weights

All animals gained weight during both weeks of the study. The individual body weights are listed in Table 4.

TABLE 4

Table Of Individual Body Weights (grams)

Dose (mg/kg)	Animal Number	Day 0	Day 7	Day 14
MALE RATS				
2000	661	215	282	312
2000	662	208	267	310
2000	663	219	276	314
2000	664	220	300	342
2000	665	206	237	258
FEMALE RATS				
2000	666	212	229	243
2000	667	212	224	241
2000	668	210	228	237
2000	669	211	229	245
2000	670	214	217	236

Necropsy Findings

Treatment-related changes seen at necropsy were limited to discoloration (black) of the hair adjacent to the application site of all animals. Hydrometra was an incidental finding noted in Rat 670. No tissue was collected for microscopic examination. A detailed record of the incidence and severity of any gross abnormalities is presented in computer-generated tables which are included in the Appendix.

DISCUSSION

In the dermal toxicity study, no mortality was noted. During the 14-day observation period, the only abnormal clinical sign was discoloration (black) of the hair immediately adjacent to the application site by the test substance. The discolored hair, seen for all animals, persisted to termination of the study and was noted at necropsy. No abnormal clinical signs were noted at the application site. No other treatment-related changes were evident at necropsy, and no tissue was collected for microscopic examination. The acute dermal LD_{50} for this test substance was greater than 2000 mg/kg for male and female rats.

CONCLUSION

Based on the dermal LD_{50} for male and female rats, the test substance was classified as, at most, slightly toxic in rats according to the criteria set forth by Hodge and Sterner (1949) and requires no toxicity classification as defined in the 18th Adaptation of the EC Classification, Packaging, and Labelling of Dangerous Substances Directive.

REFERENCES

Hodge, H.C. and Sterner, J.H. (1949). Tabulation of toxicity classes. *Am. Indust. Hyg. Quart.*, **10**, 93-96.

National Research Council (1996). *Guide for the Care and Use of Laboratory Animals.* National Academy Press. Washington, D.C.

APPENDIX

HAEL No. 97-0220
EAN 905401

PATHOLOGY REPORT

Chemical Identity: Color Developing Agent CD-3

Male and female rats exposed to 2000 mg/kg of the test substance over clipped bare skin for 24 hours, as part of an acute dermal study, were necropsied. Necropsy lesions are listed in computer-generated tables.

GROSS PATHOLOGY:

Male Rats - 2000 mg/kg exposure group: Exposure-related changes included minimal black discoloration of the hair of the back by the test substance (5/5). No signs of organ toxicity were observed. All rats survived the observation period.

Female Rats - 2000 mg/kg exposure group: Exposure-related changes included minimal black discoloration of the hair of the back by the test substance (5/5).

No signs of organ toxicity were observed. All rats survived the observation period.

Incidental findings consisted of a minor hydrometra in Rat 670.

Comments: Minimal hydrometra was an incidental finding observed in Rat 670. Hydrometra is the dilatation of the uterus with an accumulation of ovulatory intraluminal fluid during the estrus cycle of the rat.

11/12/97
Milan S. Vlaovic, D.V.M., Ph.D.

11/12/97
Reviewed by
John L. O'Donoghue, V.M.D., Ph.D.

MSV:sji
11/07/97

SUMMARY GROSS PATHOLOGY INCIDENCE TABLE - MALE RATS

GROUP	2000 MG/KG
TRACHEA	5
LUNGS	5
THYMUS	5
HEART	5
ESOPHAGUS	5
STOMACH	5
DUODENUM	5
JEJUNUM	5
ILEUM	5
CECUM	5
COLON	5
RECTUM	5
LIVER	5
KIDNEYS	5
URINARY BLADDER	5
PITUITARY GLAND	5
ADRENALS	5
PANCREAS, NOS	5
THYROID GLANDS	5
PARATHYROID GLANDS	5
SPLEEN	5
MESENTERIC LYMPH NODES	5
BONE MARROW	5
BRAIN	5
EYES	5
SALIVARY GLANDS	5
ADIPOSE TISSUE	5
SKIN, NOS	5
HAIR	5
HAIR OF BACK	
DISCOLORATION, BLACK	5
ACCESSORY SEX ORGANS (MALE)	5
EPIDIDYMIDES	5
TESTES	5

NUMBERS REPRESENT NUMBER OF TISSUES EXAMINED, OR IN THE CASE OF ABNORMAL FINDINGS, THE NUMBER OF TISSUES WITH EACH ABNORMALITY

SUMMARY GROSS PATHOLOGY INCIDENCE TABLE - FEMALE RATS

GROUP	2000 MG/KG
TRACHEA	5
LUNGS	5
THYMUS	5
HEART	5
ESOPHAGUS	5
STOMACH	5
DUODENUM	5
JEJUNUM	5
ILEUM	5
CECUM	5
COLON	5
RECTUM	5
LIVER	5
KIDNEYS	5
URINARY BLADDER	5
PITUITARY GLAND	5
ADRENALS	5
PANCREAS, NOS	5
THYROID GLANDS	5
PARATHYROID GLANDS	5
SPLEEN	5
MESENTERIC LYMPH NODES	5
BONE MARROW	5
BRAIN	5
EYES	5
SALIVARY GLANDS	5
ADIPOSE TISSUE	5
SKIN, NOS	5
HAIR	5
HAIR OF BACK	
DISCOLORATION, BLACK	5
FALLOPIAN TUBES	5
VAGINA	5
UTERUS	5
HYDROMETRA	1
OVARIES	5
CERVIX UTERI	5

NUMBERS REPRESENT NUMBER OF TISSUES EXAMINED, OR IN THE CASE OF ABNORMAL FINDINGS, THE NUMBER OF TISSUES WITH EACH ABNORMALITY

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE - MALE RATS

2000 MG/KG

ANIMAL #	661	662	663	664	665
DAYS ON TEST	14	14	14	14	14
TRACHEA	X	X	X	X	X
LUNGS	X	X	X	X	X
THYMUS	X	X	X	X	X
HEART	X	X	X	X	X
ESOPHAGUS	X	X	X	X	X
STOMACH	X	X	X	X	X
DUODENUM	X	X	X	X	X
JEJUNUM	X	X	X	X	X
ILEUM	X	X	X	X	X
CECUM	X	X	X	X	X
COLON	X	X	X	X	X
RECTUM	X	X	X	X	X
LIVER	X	X	X	X	X
KIDNEYS	X	X	X	X	X
URINARY BLADDER	X	X	X	X	X
PITUITARY GLAND	X	X	X	X	X
ADRENALS	X	X	X	X	X
PANCREAS, NOS	X	X	X	X	X
THYROID GLANDS	X	X	X	X	X
PARATHYROID GLANDS	X	X	X	X	X
SPLEEN	X	X	X	X	X
MESENTERIC LYMPH NODES	X	X	X	X	X
BONE MARROW	X	X	X	X	X
BRAIN	X	X	X	X	X
EYES	X	X	X	X	X
SALIVARY GLANDS	X	X	X	X	X
ADIPOSE TISSUE	X	X	X	X	X
SKIN, NOS	X	X	X	X	X
HAIR					
HAIR OF BACK					
DISCOLORATION, BLACK	1	1	1	1	1
ACCESSORY SEX ORGANS (MALE)	X	X	X	X	X
EPIDIDYMIDES	X	X	X	X	X
TESTES	X	X	X	X	X

KEY:N-NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY,1-MINIMAL,2-MINOR,3-MODERATE,4-SEVERE
P-PRESENT,A-ABSENT,*-SEE COMMENT REPORT, X=NORMAL BUT NOT COLLECTED

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE -FEMALE RATS

2000 MG/KG

ANIMAL #	666	667	668	669	670
DAYS ON TEST	14	14	14	14	14
TRACHEA	X	X	X	X	X
LUNGS	X	X	X	X	X
THYMUS	X	X	X	X	X
HEART	X	X	X	X	X
ESOPHAGUS	X	X	X	X	X
STOMACH	X	X	X	X	X
DUODENUM	X	X	X	X	X
JEJUNUM	X	X	X	X	X
ILEUM	X	X	X	X	X
CECUM	X	X	X	X	X
COLON	X	X	X	X	X
RECTUM	X	X	X	X	X
LIVER	X	X	X	X	X
KIDNEYS	X	X	X	X	X
URINARY BLADDER	X	X	X	X	X
PITUITARY GLAND	X	X	X	X	X
ADRENALS	X	X	X	X	X
PANCREAS, NOS	X	X	X	X	X
THYROID GLANDS	X	X	X	X	X
PARATHYROID GLANDS	X	X	X	X	X
SPLEEN	X	X	X	X	X
MESENTERIC LYMPH NODES	X	X	X	X	X
BONE MARROW	X	X	X	X	X
BRAIN	X	X	X	X	X
EYES	X	X	X	X	X
SALIVARY GLANDS	X	X	X	X	X
ADIPOSE TISSUE	X	X	X	X	X
SKIN, NOS	X	X	X	X	X
HAIR					
HAIR OF BACK					
DISCOLORATION, BLACK	1	1	1	1	1
FALLOPIAN TUBES	X	X	X	X	X
VAGINA	X	X	X	X	X
UTERUS	X	X	X	X	
HYDROMETRA					2
OVARIES	X	X	X	X	X
CERVIX UTERI	X	X	X	X	X

KEY:N-NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY,1-MINIMAL,2-MINOR,3-MODERATE,4-SEVERE
P-PRESENT,A-ABSENT,*-SEE COMMENT REPORT, X=NORMAL BUT NOT COLLECTED

FINAL REPORT

COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 **EAN: 905401**
CAS No.: 025646-71-3 **HAEL No.: 97-0220**

ACUTE EYE IRRITATION STUDY IN THE RABBIT

GUIDELINE

OECD: 405
EEC: Annex V., Test B.5

AUTHOR

Kenneth P. Shepard, B.S.

TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

LABORATORY PROJECT ID

97-0220A6

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

STUDY COMPLETION DATE

January 14, 1998

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 97-0220-1 STUDY DIRECTOR: SHEPARD,K.P. PAGE 1
ACCESSION NUMBER: 905401 01/02/98

STUDY TYPE: RABBIT EYE IRRITATION

M. L. James 1/2/98
(AUDITOR, QUALITY ASSURANCE UNIT) DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
10/13/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
10/16/97	CLINICAL SIGNS AT 72 HRS.	01/02/98
01/02/98	FINAL REPORT REVIEW	01/02/98

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

Annex 2, Organisation for Economic Cooperation and Development, Guidelines for Testing of Chemicals [C(81)30(Final)].

Kenneth P. Shepard, B.S.
Study Director

January 14, 1998
Month/Day/Year

SIGNATURE PAGE

Kenneth P. Shepard, B.S.
Study Director

January 14, 1998
Month/Day/Year

Douglas C. Topping, Ph.D.
Unit Director, Mammalian Toxicology

Jan 6, 1998
Month/Day/Year

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ABSTRACT

COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 **EAN: 905401**
CAS No.: 025646-71-3 **HAEL No.: 97-0220**

ACUTE EYE IRRITATION STUDY IN THE RABBIT

An eye irritation study was conducted with this test substance in two rabbits. After administration of a single dose of 0.1 gram of the test substance into the conjunctival sac of one eye of each animal, one treated eye was washed with distilled water. The remaining treated eye was not irrigated. Ocular lesions were graded according to OECD Guideline 405 (Annex V., Test B.5) (Grades for Ocular Lesions).

For the unwashed eye, chemosis (grade 4) of the conjunctiva and a profuse discharge was noted one hour after administration of the test substance. Signs of irritation for the unwashed eye noted between the 24-hour and 72-hour examinations included opacity (grade 1) of an area greater than one-quarter but less than one-half of the cornea, redness (grade 3) of the conjunctiva, and chemosis (grade 3) of the nictitating membrane and/or lids. A profuse discharge, which abated over time, was also noted during this period. In addition, staining of the cornea and conjunctiva were apparent for the unwashed eye when fluorescein dye was applied at the 24-hour examination. At the Day 7 examination, signs of irritation included redness (grade 2) of the conjunctiva and chemosis (grade 1) of the nictitating membrane and/or lids. Redness (grade 2) persisted through the Day 14 and Day 21 examinations. Not until the Day 28 examination was complete reversibility of the irritant effects seen for the unwashed eye. Since the test substance caused significant ocular lesions within 72 hours after exposure which persisted for more than 24 hours, a decision was made by the Sponsor not to conduct a full study (three unwashed eyes and three washed eyes).

Immediate irrigation of the eye was palliative. For the washed eye, signs of irritation noted within 72 hours of dosing included redness (grade 2) of the conjunctiva and chemosis (grade 2) of the nictitating membrane and/or lids. A profuse discharge was noted only at the one-hour examination. No staining was apparent for the washed eye when fluorescein dye was applied. By the Day 7 examination, the irrigated eye appeared clinically normal.

Based on the results of this study, the test substance was classified as irritating to eyes as defined in the 18th Adaptation of the EC Classification, Packaging, and Labeling of Dangerous Substances Directive.

STUDY AND TEST SUBSTANCE INFORMATION

Testing Facility

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

Project Participants

Study Director:	Kenneth P. Shepard, B.S.
Principal Investigator:	John W. Mosher, B.S.

Sponsor

Eastman Chemical Company
P.O. Box 1994
First American Center
Kingsport, TN 37662-5394

Sponsor's Representative:
Karen R. Miller, Ph.D.

Test Substance Characterization

Chemical Identity:	Color Developing Agent CD-3
Chemical Name:	N-(2-((4-Amino-3-methylphenyl)ethylamino) ethyl)methanesulfonamide sulfate (2:3) monohydrate
CAS No.:	025646-71-3
PM No.:	01578-00
EAN:	905401
HAEL No.:	97-0220
SRID or Lot No.:	75168
Physical State and Appearance:	Solid, Off-white powder
Source of Test Substance:	Eastman Chemical Company
Laboratory Project ID:	97-0220A6

Study Dates

Study Initiation Date:	October 10, 1997
Experimental Start Date:	October 13, 1997
Experimental Completion Date:	November 10, 1997

PURPOSE

The purpose of the study was to determine the potential of the test substance to cause primary ocular irritation.

MATERIALS AND METHODS

Test system

Two albino rabbits (Hra:(NZW)SPF) obtained from Covance Research Products Inc. (Denver, PA) were assigned to the study. The rabbits were young adults (at least three months old). Rabbits were chosen for this study because they are a common representative species for eye irritation studies. The adult albino rabbit is the preferred species recommended for use in the OECD guideline.

Husbandry

Housing

Animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited vivarium in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The rabbits were singly housed in suspended, stainless-steel mesh cages. Cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed every other day.

Environmental Conditions

The study room was maintained at 18-22°C and 46-62% relative humidity. A photoperiod of 12 hours light from 6 a.m. to 6 p.m. was maintained.

Acclimation Period

The animals were isolated upon arrival and allowed to acclimate for a period of 5 days. Animals were judged to be healthy prior to testing.

Feed

Certified High Fiber Rabbit Diet (PMI® #5325) was available *ad libitum*. Feed containers were cleaned weekly and refilled at least once a week. No known contaminants which would interfere with the outcome of this study were present in the feed. Analyses of feed are maintained on file within the testing laboratory.

Husbandry, continued

Water

Water was available *ad libitum* through an automatic watering system. The source of the water was the local public water system. There have been no contaminants identified in previous water analyses that would be expected to interfere with the conduct of the study. Semiannual analyses of water are maintained on file within the testing laboratory.

Identification

Upon arrival, all rabbits were identified by uniquely-numbered ear tags. Cage cards contained the study-specific animal number and the ear tag number.

Experimental Design

Test Procedures

This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals: Guideline 405, Acute Eye Irritation/Corrosion; and European Economic Community (EEC): Annex V., Test B.4, Acute Toxicity (Eye Irritation).

Identification Numbers of Animals Used and Group Distribution

Animals were distributed into groups as described in Table 1.

TABLE 1 (Distribution Of Animals)

Group	Number of Animals	Animal Numbers
Not Irrigated	1	349
Irrigated	1	352

Preparations

Both eyes of each rabbit selected for the study were tested with fluorescein dye and examined within 24 hours of administering the test substance. Animals showing eye irritation, ocular defects, or pre-existing corneal injury were not used.

Experimental Design, continued

Preparation of Test Substance

The test substance, a solid, was administered as received.

Test Substance Exposure

A single dose of 0.1 gram of the test substance was placed into the conjunctival sac of one eye (left) of each animal. One of the treated eyes was immediately washed with running distilled water; the other treated eye was not irrigated.

Control Substance

No control substance was used. The untreated eye (right) of each animal served as a control for the test.

Clinical Observations

Eyes were observed immediately after instillation of the test substance and 1, 24, 48, and 72 hours, and 7, 14, 21, and 28 days thereafter. Observations included indications of immediate sensory irritation and estimations of edema and redness of the adnexal structures. Also evaluated were effects on the iris, the presence of corneal opacity, and any discharge from the eye. Eyes were treated with a 2% ophthalmic solution of fluorescein at 24 hours and observed for staining.

Grading the Irritant Response

The eyes were examined and grades of ocular reactions recorded for each animal at all observation periods. Ocular lesions were graded and scored as described in Table 2.

TABLE 2 (Grading Of Ocular Lesions[1])

Cornea	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous area, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4

[1] Graded as described in OECD Guideline 405 (Annex V., Test B.5) (Grading for Ocular Lesions)

continued on next page

Experimental Design, continued

Grading the Irritant Response, continued

TABLE 2 (Grading Of Ocular Lesions[1]), continued

Area of Corneal Opacity	
One-quarter or less, but not zero	1
Greater than one-quarter up to one-half	2
Greater than one-half up to three-quarters	3
Greater than three quarters up to the whole area	4
Iris	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal hyperaemia, or injection, any of these or combinations of any thereof, iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2
Conjunctivae	
Redness: refers to palpebral and bulbar conjunctivae excluding cornea and iris	
Blood vessels normal	0
Some blood vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
Chemosis: lids and/or nictitating membranes	
No swelling	0
Any swelling above normal (including nictitating membranes)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

[1] Graded as described in OECD Guideline 405 (Annex V., Test B.5) (Grading for Ocular Lesions)

Grading Other Clinical Observations

Other serious eye lesions, signs of abnormality, or toxic effects were graded and scored as described in Table 3.

TABLE 3 (Grading Of Other Clinical Observations)

Degree of Severity	
Slight	1
Moderate	2
Severe	3

Body Weight Determinations

Animals were not weighed as weights are not critical to this study.

Necropsy

No necropsies were conducted at the conclusion of the observation period.

Data Storage

The final report, data sheets, all nonperishable raw data, and an aliquot of the test substance have been stored in the testing facility archive managed under GLP-mandated conditions.

Protocol and Standard Operating Procedure Deviations

The were no SOP or protocol deviations during the study.

RESULTS

Observations for Irritation

The treated eye of each animal was examined for signs of irritation at 1, 24, 48, and 72 hours, and 7, 14, 21, and 28 days after instillation of the test substance. Observations of corneal opacity, iris effects, redness, and chemosis are listed in Table 4.

TABLE 4 (Ocular Irritation)

Animal Number	Irrigated	Corneal Opacity, Iris Effects, Redness, Chemosis [1]			
		1 Hour	24 Hours	48 Hours	72 Hours
349	No	0,0,0,4	1,0,2,3	0,0,2,3	0,0,3,3
352	Yes	0,0,0,2	0,0,2,1	0,0,2,1	0,0,2,0

Animal Number	Irrigated	Corneal Opacity, Iris Effects, Redness, Chemosis [1]			
		7 Days	14 Days	21 Days	28 Days
349	No	0,0,2,1	0,0,2,0	0,0,2,0	0,0,0,0
352	Yes	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0

[1] Graded as described in OECD Guideline 405 (Annex V., test B.5) (Grades for Ocular Lesions).

Description of Serious Lesions and Other Observations not Previously Described

For the unwashed eye, other observations included a profuse discharge at the one-hour and 24-hour examinations; a moderate discharge at the 48-hour examination, a slight discharge at the 72-hour examination, and fluorescein staining of the conjunctiva and cornea at the 24-hour examination. For the washed eye, other observations were limited to a profuse discharge at the one-hour examination. No fluorescein staining was noted for the irrigated eye. No toxic effects were noted during the study.

Effects of Immediate Washing

Immediate irrigation of the eyes was palliative.

DISCUSSION

Prior to the eye irritation study, an acute dermal irritation study was performed in an attempt to avoid testing substances that would produce significant irritation in the eyes of rabbits. Results from this study determined that the test substance would not be classified as irritating to skin.

Therefore, an eye irritation study was conducted in which, initially, only two eyes (one unwashed and one washed) were tested. The severity of ocular lesions were graded according to OECD Guideline 405 (Annex V Test B.5).

In the 72 hours following instillation of the test substance to the unwashed eye, redness (grade 3), chemosis (grade 4), corneal opacity (grade 1), and a profuse discharge were noted. In addition, staining of the cornea and conjunctiva were apparent for the unwashed eye when fluorescein dye was applied at the 24-hour examination. These changes were sufficient to characterize the response as significant irritation, and therefore, a decision was made by the Sponsor not to conduct a full study (three unwashed eyes and three washed eyes). Complete reversibility of the irritant effects for the unwashed eye was noted only at the Day 28 examination.

Immediate irrigation of the eye was palliative. For the washed eye, signs of irritation were limited to redness (grade 2) and chemosis (grade 2). A profuse discharge was also seen one hour after administration of the test substance. No staining was evident when fluorescein dye was applied. By Day 7 of the study, the washed eye appeared normal.

CONCLUSION

Based on the results of this study, the test substance was classified as irritating to eyes as defined in the 18th Adaptation of the EC Classification, Packaging, and Labeling of Dangerous Substances Directive.

905401

188552J

TX-85-74

Acute Toxicity of CD-3

HAEL No. 85-0086 Acc. No. 905401

Report prepared by M. W. Barrows, B.S.

Supervisor of Toxicological Sciences C. J. Terhaar, Ph.D.

Study Request Date August 19, 1985

Date Work Started September 3, 1985

Date Work Completed October 7, 1985

Report Issue Date December 5, 1985

Toxicological Sciences Section

Health and Environment Laboratories

Eastman Kodak Company

Rochester, N.Y, 14650

188552J
TX-85-74

SUMMARY OF ACUTE TOXICITY STUDIES

Chemical CD-3

Acc. No. 905401 HAEL No. 85-0086 Source I.D. 40018

GAVAGE LD_{50} (mg/kg) Rats (M) 400 (209-765) Rats (F) 152 (98-235)

INHALATION LC_{50} (mg/m^3, ppm) Rats (M) -- Rats (F) --

Dose levels tested: 50, 100, 200, 400, 800 mg/kg

No. Rats/dose: 5 (Females only at 50 mg/kg, Males only at 800 mg/kg)

Vehicle: Distilled water

Initial Body weight range (g): (M) 214-274 (F) 160-209

Strain: CRL: CD® (SD)BR

Method of calculation: Weil method

Test SOP No.: TA 300, TA 120

REMARKS: Clinical signs of toxicity included slight-severe weakness, prostration, tremors, ataxia, rough coat and anorexia. Lesions related to the material were observed in the heart and liver upon necropsy of animals dying spontaneously. Histologic lesions were also observed in the liver and heart.

DERMAL LD_{50} (mg/kg) Guinea Pigs (M) _____ Guinea Pigs (F) _____

Dose levels tested:

No. Guinea Pigs/dose:

Vehicle:

Initial Body weight range (g): (M) _____ (F) _____

Strain: CRL (HA)BR Hartley

Method of calculation: Weil method

Test SOP No.: TA 310, TA 160, TA 120

REMARKS: Not done

188552J
TX-85-74

GUINEA PIG SKIN IRRITATION

24 hr. Occluded Single Dose

Slight Moderate Strong Severe

Dose levels tested:

No. guinea pigs/dose:

Vehicle:

Initial Body weight range (g):

Strain: CRL (HA)BR Hartley

Test SOP No.: TA 320, TA 160

Sex:

REMARKS: Not done

Open Repeated

Dose levels tested:

No. of doses: No. of days:

No. guinea pigs/dose:

Vehicle:

Initial Body weight range (g):

Strain: CRL: (HA)BR Hartley

Test SOP No.: TA-170

Sex:

REMARKS: Not done

Acute Skin Irritation

Slight

Moderate

Strong

Estimated Corrosivity

No

Questionable

Yes

Skin Absorption -- (Based on Dermal LD_{50} and Skin Irritation studies)

188552J
TX-85-74

GUINEA PIG SENSITIZATION

Estimated Human Risk

KODAK METHOD

No. responding: None Slight Moderate Strong

Induction dose level: 0.05 mL of a ___% solution of compound in Freund's Complete Adjuvant — Low

No. of doses: — Moderate

No. guinea pigs/dose: — High

Challenge concentration:

Challenge vehicle:

Initial Body weight range (g):

Strain: CRL (HA)BR Hartley

Test SOP No.: TA-180

Sex:

REMARKS: Not done

BUEHLER METHOD

No. responding: None Slight Moderate Strong

No. guinea pigs per dose:

Induction

Preparation:
No. of weekly doses:

Challenge

Preparation:

Initial Body weight range (g):

Strain: CRL (HA)BR Hartley

Test SOP No.: TA 350

Sex:

REMARKS: Not done

188552J
TX-85-74

RABBIT EYE IRRITATION

No. responding:	Slight	Moderate	Strong	Severe	Fluorescein stain Cornea	Fluorescein stain Adnexa
Unwashed						
Washed						

Dose level tested:

No. of doses:

No. rabbits/dose:

Vehicle: none

Washing agent: distilled water

Body weight range:

Strain: New Zealand white

Test SOP No.: TA 330, TA 150

Sex:

Acute Eye Irritation
Slight
Moderate
Strong

REMARKS: Not done

Comments: All animals were identified by metal ear tags/cage number. All specimens, raw data, and the final report of this work are stored in the archives of the Health and Environment Laboratories. Only limited analyses have been completed on the strength, purity, composition, stability, uniformity, and concentration of the test material. Professionals involved in this study other than the study director included: M. S. Vlaovic, D.V.M., Ph.D., Laboratory Animal Medicine and John L. O'Donoghue, V.M.D., Ph.D., Pathologist. Deviations from approved protocols or standard operating procedures included:

Signature of Study Director for Acute Studies:

Douglas C Joppi Date 4 Dec 1985

Signature of Supervisor, Toxicological Sciences:

C J Perkaar Date 5 Dec. '85

ACUTE TOXICITY - LD_{50}

ACCESSION NO.	SOURCE REF. I.D. NO.	HS & HFL NO.
905401	40018	85-0086

COMPOUND: CD-3

SOURCE: TEC

SOLUTION: 10% in H_2O

P.O. FASTED RATS

DATE: 9/23/85

DOSE mg/Kg	NO. RATS (M, F)	NO. DEATHS (M, F)	TIME OF DEATH	1 WEEK WEIGHT CHANGE	2 WEEK WEIGHT CHANGE
50	0 - 5	--, 0	--	--, 5+	--, 5+
100	5 - 5	0, 1	Day 1	5+, 4+	4+, 4+
200	5 - 5	1, 3	Day 1, 3	4+, 2+	4+, 2+
400	5 - 5	2, 5	Day 1 and 2	3+, --	3+, --
800	5 - 0	4, --	Day 1	1+, --	1+, --
1600					
3200					

SUMMARY

SIGN	DOSE (mg/Kg)	TIME	SEX
Normal	50 to 800	Immed.-Day 2 to Day 14	M,F
Sl. Weakness	50 to 800	1 hr. to Day 3	M,F
Mod. Weakness	200, 400, and 800	3 hrs. to Day 2	M,F
Sev. Weakness	400, 800	3 1/2 hrs. to Day 2	M,F
Prostration	200, 400 and 800	3 1/2 hrs. to Day 3	M,F
Tremors	200	40 min.	F
Ataxia	400	3 hrs. to Day 2	F
Rough coat	400, 800	1 hr. to Day 2	M,F
Pallor	400	1 hr.	M
Anorexia	50, 400 and 800	Day 2	M,F

Remarks: Moderately toxic by the oral route. Lesions related to the material were observed in the heart and liver upon necropsy of animals dying spontaneously. Histologic lesions were also observed in the liver and heart of these animals.

LD_{50} Males: 400 mg/kg - C.I. 209-765

Females: 152 mg/kg - C.I. 98-235

Q.A. INSPECTION STATEMENT
(CFR 58.35(B)(7))

STUDY: 85-0086-1 STUDY DIRECTOR: TOPPING,D.C.
ACCESSION NUMBER: 905401

STUDY TYPE: ACUTE TOXICITY REPORT

M L James 11/5/85
(AUDITOR, QUALITY ASSURANCE UNIT) DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF THE HAEL, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECTION DATES	STUDY TYPE/PHASE(S) INSPECTED	STATUS REPORT DATES
09/03/85	ACUTE LD50 PROTOCOL APPENDIX SUBMISSION	
09/09/85	ACUTE LD50 PROTOCOL APPENDIX SUBMISSION CLINICAL SIGNS AT 1 HR. NECROPSY	09/09/85
11/05/85	ACUTE TOXICITY REPORT FINAL REPORT REVIEW	11/05/85

>>

FINAL REPORT

"EASTMAN" COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 EAN: 905401
CAS No.: 025646-71-3

ACUTE TOXICITY STUDY IN THE RAT (INTRAPERITONEAL INJECTION OF TEST SUBSTANCE)

AUTHOR

Kenneth P. Shepard, B.S.

TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

REPORT DATE

August 7, 1997

"EASTMAN" COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 EAN: 905401
CAS No.: 025646-71-3

ACUTE TOXICITY STUDY IN THE RAT (INTRAPERITONEAL INJECTION OF TEST SUBSTANCE)

An acute toxicity study was conducted in rats by injecting the test substance into the intraperitoneal cavity. The study was initiated on March 1, 1955. All data from this study were stored in the testing facility archive managed under GLP-mandated conditions. This report was prepared from the archived raw data.

For this study, seven rats were used. Prior to administration of the test substance, individual body weights were recorded. Initially, five rats received a single dose of either 25, 50, 100, 200, or 400 mg/kg of the test substance prepared as a 10% solution in water. Rats assigned to the 100, 200, and 400 mg/kg dose groups died on the day of dosing. The rat at the 50 mg/kg dose level died seven days after dosing and the rat at the 25 mg/kg dose level died 11 days after administration of the test substance. Due to mortality seen at all dose levels, two additional rats administered a single dose of either 10 or 25 mg/kg of the test substance were added to the study. The rat that received 10 mg/kg of the test substance died three days prior to termination of the study while the rat administered 25 mg/kg of the test substance survived a 14-day period and gained weight during the study.

The dose level, animal number, individual body weights, volume of test substance administered, and the day of death are listed in Table 1. Observations prior to death included weakness, ataxia, unconsciousness, gasping, and mild convulsions. Observations noted for the single rat at the 25 mg/kg dose level which survived were limited to transient weakness and apparent disorientation.

TABLE 1

Dose (mg/kg)	Animal Number	Start Body Weight (g)	End Body Weight (g)	Volume (mL)	Time Of Death
10	337	304	------	0.03	Day 11
25	338	305	357	0.08	Not Applicable
25	123	307	------	0.08	Day 11
50	124	306	------	0.15	Day 7
100	125	267	------	0.27	Day of Dosing
200	126	309	------	0.62	Day of Dosing
400	127	279	------	1.12	Day of Dosing

DISCUSSION

An acute toxicity study was conducted in which rats were administered 10, 25, 100, 200, or 400 mg/kg of the test substance by the intraperitoneal route. Mortality was 100% for rats at dose levels of 10, 50, 100, and 400 mg/kg and 50% for rats at a dose level of 25 mg/kg. The acute LD_{50} for this test substance administered by intraperitoneal injection to rats was less than 10 mg/kg.

CONCLUSION

The test substance was considered to be highly toxic in rats when administered by the intraperitoneal route.

MAR 10 1986

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Addendum to Acute Toxicity of CD-3

HAEL No. 85-0086 Acc. No. 905401

Report prepared by D. C. Topping, Ph.D.

Supervisor of Toxicological Sciences C. J. Terhaar, Ph.D.

Date of Study Initiation November 20, 1985

Date of Study Completion February 28, 1986

Toxicological Sciences Section

Health and Environment Laboratories

Eastman Kodak Company

Rochester, N.Y. 14650

188552J
TX-85-74

SUMMARY OF ACUTE TOXICITY STUDIES

Chemical CD-3

Acc. No. 905041 HAEL No. 85-0086 Source I.D. 40018

GAVAGE LD_{50} (mg/kg) Rats (M) 400 (285-562) Rats (F) 246 (141-429)

INHALATION LC_{50} (mg/m^3, ppm) Rats (M)_____ Rats (F)_____

Dose levels tested: 50, 100, 200, 400, 800

No. Rats/dose: 5 males, 5 females

Vehicle: distilled water

Initial Body weight range (g): (M) 134-190 (F) 158-189

Strain: CRL: CD® (SD)BR

Method of calculation: Weil method

Test SOP No.: TA 300, TA 120

REMARKS: The material was moderately toxic by the oral route. Clinical signs included slight to severe weakness in all dose groups, convulsions in the 800 mg/kg group, brown urine in all dose groups for the first three days of the study and rough hair coats in all survivors. Lesions observed grossly attributable to the toxic action of the material were limited to gastric hemorrhage and edema. Microscopic lesions indicative of target organ toxicity were found in males dosed with 400 mg/kg and 200 mg/kg and surviving to the end of the experiment, and were found only in the kidneys. No lesions attributable to the test material were observed in the hearts or brains of either sex or the kidneys of the females. Other gross and microscopic lesions observed were considered to be agonal in nature or of spontaneous origin. The reports of the veterinary pathologist are appended to this report.

Comments: All animals were identified by metal ear tags/cage number. All specimens, raw data, and the final report of this work are stored in the archives of the Health and Environment Laboratories. Only limited analyses have been completed on the strength, purity, composition, stability, uniformity, and concentration of the test material. Professionals involved in this study other than the study director included: Milan S. Vlaovic, D.V.M., Ph.D., Laboratory Animal Medicine, John L. O'Donoghue, V.M.D., Ph.D., Pathologist. Deviations from approved protocols or standard operating procedures included: The weight range in the male animals exceeded ± 20%.

Signature of Study Director for Acute Studies:

Douglas C. Keppy Date 28 Feb 1986

Signature of Supervisor, Toxicological Sciences:

C J Nerhaar Date 28 Feb. 86

ACUTE TOXICITY - LD_{50}

ACCESSION NO.	SOURCE REF. I.D. NO.	HS & HFL NO.
905401	40018	85-0086

COMPOUND: CD-3

SOURCE: TEC

SOLUTION: 10 gr. cpd. in 90 mL distilled H_2O

P.O. FASTED RATS

DATE: 12/4/85

DOSE mg/Kg	NO. RATS (M, F)	NO. DEATHS (M, F)	TIME OF DEATH	1 WEEK WEIGHT CHANGE	2 WEEK WEIGHT CHANGE
50	5 - 5	0 - 0	--	5+ - 5+	5+ - 5+
100	5 - 5	0 - 1	Day 1	5+ - 4+	5+ - 4+
200	5 - 5	0 - 2	Day 1	5+ - 3+	5+ - 3+
400	5 - 5	2 - 3*	Day 1,3 - Day 1	3+ - 1+	3+ - 1+
800	5 - 5	5 - 5	Day 1,3 - Day 1	--	--
1600					
3200					

*3 due to chemical; 1 additional death unrelated to treatment

SUMMARY

SIGN	DOSE (mg/Kg)	TIME	SEX
Weakness	50,100,200,400	Day 1-3	M,F
Slight to Moderate	800	Day 1-3	M
Weakness Severe	800	Day 1	M,F
Convulsions	800	Day 1	M
Brown Urine	Survivors of all doses	Day 1-3	M,F
Rough Coat	Survivors of all doses	Day 1-14	M,F

REMARKS

See attached sheet for remarks section.

LD_{50} Males: _____ mg/kg - C.I. _____

Females: _____ mg/kg - C.I. _____

KPD-0153-412 (3/83)

188552J
TX-85-74

Remarks for Acute Oral LD_{50} on 85-0086 (CD-3)

Remarks: Moderately toxic by the oral route. Clinical signs included slight to severe weakness in all dose groups and convulsions in the 800 mg/kg dose group. Brown urine was observed from survivors of all doses during the first several days, and all survivors had rough hair coats for the duration of the test. Gross lesions directly attributable to the toxic action of the material were limited to gastric hemorrhage and edema. Microscopic lesions associated with the test material were not observed in hearts or brains, but at 400 and 200 mg/kg, some males surviving to the 14th day demonstrated changes in the kidneys indicative of chronic inflammation and were considered compound-related.

LD_{50} males 400 mg/kg C.I. 285-562

LD_{50} females 246 mg/kg C.I. 141-429

Douglas C. Topping Feb 24, 1986
Douglas C. Topping

Acc. No. 905401
Study No. 85-0086

PATHOLOGY REPORT

Compound: CD-3 (This report covers rats which did not survive to the end of the study.)

Male rats given 400 or 800 mg/kg of the test compound and female rats given 100, 200, 400, or 800 mg/kg of the test compound as part of an LD_{50} study, were necropsied. Necropsy lesions are listed on Table 1. All of the necropsied rats died on the same day they were dosed except for Rat 139 which died two days later. Therefore, all of the stomachs (except for Rat 139) contained the test chemical in their lumena and four rats had the chemical present in the small intestine. On necropsy of Rat 139, four large uroliths were found in the bladder obstructing the urethra. Necropsy lesions in the kidneys and stomach and microscopic lesions in the urinary bladder were consistent with long-standing urolithiasis and death due to uremia following uretheral obstruction. The death of Rat 139 should not be considered in the calculation of an LD_{50} for this compound.

A number of lesions were found in the remaining rats. Some of these were probably related to exposure to the test compound. These include: lungs which did not collapse when the thorax was opened; pulmonary congestion and hemorrhage; gastric hemorrhage (Rat 145 only); gastric edema; renal congestion; hepatic congestion; perioral or perinasal wetness, blood or discoloration; hyperemia of the small intestine; blood in the thorax; splenic pallor; and cardiac hemorrhage. None of these changes were indicative of a specific organ effect due to the test chemical except for gastric hemorrhage and edema which were probably due to chemical irritation. The rest of the changes were probably agonal in nature. Lesions not specifically mentioned above were considered spontaneous and unrelated to chemical exposure.

The heart, kidneys, and brains from all necropsied rats and the urinary bladder from Rat 139 were examined microscopically. No compound-related lesions were identified. See Table 2 for a listing of microscopic lesions.

1/3/86

John L. O'Donoghue, V.M.D., Ph.D.

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE

TABLE 1

GROUP:	Males 400 mg/kg				Males 800 mg/kg						Females 100 mg/kg				Females 200 mg/kg				Females 400 mg/kg					Females 800 mg/kg				
COMPOUND NUMBER: LD_{50} 85-0086	R138	R139			R142	R143	R144	R145			R247				R254	R255			R256	R258	R259	R260		R261	R262	R263	R264	R265
Lungs																												
focal pneumonitis																												2
did not collapse															P													
congestion																				2				2				
hemorrhage																								2				
Thymus																												
hemorrhage	2				1		1	1							1							1			2			
Stomach																												
chemical in lumen	P				P	P	P	P							P	P			P	P	P	P		P	P		P	P
glandular mucosa, hemorrhage		2						2																				
glandular mucosa, edema																				2					2			
glandular mucosa, discolored yellow																					2							
Kidneys																												
unilateral hydronephrosis	3							2														3						
bilateral hydronephrosis		2																										
congestion		3																										
Liver																												
congestion					2	3		2								2				2		2			2			
Urinary bladder																												
urolithiasis		P																										
Male accessory sex glands																												
atrophy	3																											
Body surfaces																												
perioral or perinasal area wet	P			P															P					P				
blood around nose	P																											
perioral area discolored purple																										P		

Key: P-present A-absent N-normal 1-minimal 2-minor 3-moderate 4-severe

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE

TABLE 1 (cont.)

COMPOUND NUMBER: LD_{50} 85-0086

GROUP:	Males 400 mg/kg				Males 800 mg/kg						Females 100 mg/kg				Females 200 mg/kg				Females 400 mg/kg				Females 800 mg/kg					
	R138	R139			R142	R143	R144	R145			R247				R254	R255			R256	R258	R259	R260		R261	R262	R263	R264	R265
Small intestine																												
hyperemia					P																							
chemical in lumen						P														P							P	P
Thorax																												
contained unclotted blood					P																							
Spleen																												
pale					P										P							P		P				
Uterus																												
hydrometra																2								2	2			
Heart																												
hemorrhage																			1									
Autolysis		4																										
No necropsy lesions											P																	

Key: P-present A-absent N-normal 1-minimal 2-minor 3-moderate 4-severe

INDIVIDUAL ANIMAL HISTOPATHOLOGY INCIDENCE TABLE

TABLE 2

GROUP:	Males													Females															
	400 mg/kg				800 mg/kg					100 mg/kg				200 mg/kg				400 mg/kg					800 mg/kg						
COMPOUND NUMBER: LD_{50} 85-0086	R138	R139			R142	R143	R144	R145		R247				R254	R255			R256	R258	R259	R260		R261	R262	R263	R264	R265		
Heart	N				N	N		N						N	N			N	N	N	N		N	N	N	N	N		
focal chronic inflammation		1					1																						
hemorrhage										2																			
Kidneys					N		N			N				N	N				N	N			N	N	N	N	N		
focal chronic inflammation	3	1				1												1											
unilateral acute pyelonephritis	3							2																					
unilateral hydronephrosis																					3								
bilateral hydronephrosis		2																											
hyperplasia pelvic epithelium		P																											
autolysis		4																											
Brain	N				N	N	N	N		N				N	N			N	N	N	N		N	N	N	N	N		
congestion		2																											
autolysis		3																											
Urinary bladder																													
papillary hyperplasia		4																											
edema		4																											
hemorrhage		3																											
congestion		4																											
acute inflammation		2																											

Key: P-present A-absent N-normal 1-minimal 2-minor 3-moderate 4-severe

Study No. 85-0086
Acc. No. 905401

PATHOLOGY REPORT

Compound: CD-3

Male and female rats given 50, 100, 200 or 400 mg/kg of CD-3 as part of an LD_{50} were necropsied. Rat 141, 800 mg/kg male, died 3 days post-dose and was not necropsied because of autolysis. All the other animals covered in this report survived the two-week observation period and were euthanatized.

Necropsy lesions are listed in Tables 1 and 3. None of the necropsy lesions were considered compound-related.

The lungs, heart, kidneys, brain and selected gross lesions were processed for microscopic examination. Histopathologic lesions are listed in Tables 2 and 4. The only compound-related histopathologic lesion was found in male rat kidneys from animals receiving the 400 mg/kg group and in one male from the 200 mg/kg group (Rat 135). This lesion consisted of patchy areas of the renal cortex containing dilated tubules with basophilic cytoplasm. The interstitial spaces in these areas were lightly infiltrated with chronic inflammatory cells, particularly lymphocytes. These morphologic changes are typical of tubule regeneration. Similar lesions, but of lesser severity, may occur in untreated animals. The remaining histopathologic lesions were considered of spontaneous origin. No compound-related lesions were observed in female animals.

John L. O'Donoghue 1/24/86.

John L. O'Donoghue, V.M.D., Ph.D.

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE - MALES

Table 1

LD_{50} GROUP:	50 mg/kg					100 mg/kg					200 mg/kg				
COMPOUND NUMBER: 85-0086	R 121	R 122	R 123	R 124	R 125	R 126	R 127	R 128	R 129	R 130	R 131	R 132	R 133	R 134	R 135
No necropsy lesions			N		N	N				N					N
Autolysis - no necropsy															
Lungs															
focal red discoloration	1	1		1			1				1		1		
focal white discoloration								1				1			
Kidneys															
pale	2											1	2		
unilateral hydronephrosis	1														
Liver															
yellow discoloration							1								
Spleen															
enlarged									3		2	2			
dark									3		2	3			
Thymus															
hemorrhage														1	

LD_{50} GROUP:	400 mg/kg					800 mg/kg									
COMPOUND NUMBER: 85-0086	R 136	R 137	R 140	R	R	R 141	R	R	R	R	R	R	R	R	R
No necropsy lesions			N												
Autolysis - no necropsy						P									
Lungs															
focal red discoloration		1													
focal white discoloration															
Kidneys															
pale		2													
unilateral hydronephrosis															
Liver															
yellow discoloration															
Spleen															
enlarged															
dark	3	3													
Thymus															
hemorrhage	1	1													

Key: P-present A-absent N-normal 1-minimal 2-minor 3-moderate 4-severe

INDIVIDUAL ANIMAL HISTOPATHOLOGY INCIDENCE TABLE - MALES

Table 2

LD50 GROUP:	50 mg/kg					100 mg/kg					200 mg/kg				
COMPOUND NUMBER: 85-0086	R 121	R 122	R 123	R 124	R 125	R 126	R 127	R 128	R 129	R 130	R 131	R 132	R 133	R 134	R 135
Lungs									N		N				
atelectasis	2							2							
focal hemorrhage	1														
perivascular cuffing	1	2	1	1	1	1	1	2		1		1	1		1
chronic bronchitis		3		2	1	1		2		1				1	1
interstitial inflammation			1					2							
Heart	N	N	N	N	N	N	N		N	N		N	N	N	N
focal chronic inflammation								1			1				
Kidneys			N						N	N				N	
basophilic tubules	1					1		1			1				2
dilated tubules											1				2
unilateral hydronephrosis	3														
focal chronic inflammation		2		2	1						1	1	1		1
hyalin droplet formation					2						1				
cyst							P								
Brain	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Liver							N								
Spleen											N	N			
inc. extramed. hematopoiesis									P						

GROUP:	400 mg/kg														
LD50 COMPOUND NUMBER: 85-0086	R 136	R 137	R 140	R	R	R	R	R	R	R	R	R	R	R	R
Lungs		N													
atelectasis			2												
focal hemorrhage															
perivascular cuffing			1												
chronic bronchitis	1														
interstitial inflammation															
Heart	N	N	N												
focal chronic inflammation															
Kidneys															
basophilic tubules	2	2	2												
dilated tubules	2	2	1												
unilateral hydronephrosis															
focal chronic inflammation	2	2	2												
hyalin droplet formation															
cyst															
Brain	N	N	N												
Liver															
Spleen															
inc. extramed. hematopoiesis		P													

Key: P-present A-absent N-normal 1-minimal 2-minor 3-moderate 4-severe

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE - FEMALES

Table 3

LD_{50} GROUP: 50 mg/kg, 100 mg/kg, 200 mg/kg

COMPOUND NUMBER: 85-0086

	50 mg/kg					100 mg/kg					200 mg/kg				
	R 241	R 242	R 243	R 244	R 245	R 246	R 248	R 249	R 250	R	R 251	R 252	R 253	R	R
No necropsy lesions							N					N			
Lungs															
focal red discoloration	1	1	1	1	1	1		1	1		1		1		
focal white discoloration	1	1	1	1	1	1		1			1				
Kidneys															
soft texture						2									
Liver															
yellow discoloration		2									2		1		
Spleen															
small													3		
Thymus															
hemorrhage		1													
Uterus															
hydrometra					2										

LD_{50} GROUP: 400 mg/kg

COMPOUND NUMBER: 85-0086

	R 257	R	R	R	R	R	R	R	R	R	R	R	R	R	R
No necropsy lesions	N														
Lungs															
focal red discoloration															
focal white discoloration															
Kidneys															
soft texture															
Liver															
yellow discoloration															
Spleen															
small															
Thymus															
hemorrhage															
Uterus															
hydrometra															

Key: P-present A-absent N-normal 1-minimal 2-minor 3-moderate 4-severe

INDIVIDUAL ANIMAL HISTOPATHOLOGY INCIDENCE TABLE - FEMALES Table 4

LD50 GROUP:	50 mg/kg					100 mg/kg					200 mg/kg				
COMPOUND NUMBER: 85-0086	R 241	R 242	R 243	R 244	R 245	R 246	R 248	R 249	R 250	R	R 251	R 252	R 253	R	R
Lungs					A										
atelectasis		2													
perivascular cuffing	2	2	2	2		2	2	2	1		1	2			
chronic bronchitis	2	2	2	2		3	2	3	2		2	2	1		
interstitial inflammation		3	1	1		3	2				2				
Heart	N	N	N		N		N	N			N	N	N		
focal chronic inflammation				1		2			2						
Kidneys	N		N				N	N	N			N			
basophilic tubules											1				
focal chronic inflammation				1		1					1				
cyst		P		P		P							P		
focal fibrosis				1											
mineralization					2										
Brain	N	N	N	N	N	N	N	N	N		N	N	N		
Liver											N				
focal chronic inflammation		1											1		
Spleen													N		

LD50 GROUP:	400 mg/kg														
COMPOUND NUMBER: 85-0086	R 257	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Lungs															
atelectasis															
perivascular cuffing	1														
chronic bronchitis	2														
interstitial inflammation															
Heart	N														
focal chronic inflammation															
Kidneys	N														
basophilic tubules															
focal chronic inflammation															
cyst															
focal fibrosis															
mineralization															
Brain	N														
Liver															
focal chronic inflammation															
Spleen															

Key: P-present A-absent N-normal 1-minimal 2-minor 3-moderate 4-severe

Q.A. INSPECTION STATEMENT
(CFR 58.35(B)(7))

STUDY: 85-0086-2 STUDY DIRECTOR: TOPPING,D.C.
ACCESSION NUMBER: 905401

STUDY TYPE: ACUTE LD50

(AUDITOR, QUALITY ASSURANCE UNIT) DATE 2/28/86

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF THE HAEL, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECTION DATES	STUDY TYPE/PHASE(S) INSPECTED	STATUS REPORT DATES
11/20/85	ACUTE LD50 PROTOCOL APPENDIX SUBMISSION	
02/28/86	ACUTE LD50 FINAL REPORT REVIEW	02/27/86

Acc. No. [illegible]01 AQUATIC TOXICITY REPORT - E.K. CO - HEALTH AND SAFETY LABORATORY HSL 7[illegible]-243

Chemical: CD-3 Source: T. Dagon 905401

Animals* No. and Species	Cond.**	Date	Dose Range mg or µl/l	Test Start Temp.	Test Start DO	Test Start pH	Test End Temp.	Test End DO	Test End pH	LT_{50} hours	Survivors at test end (hours)
	SL		mg/l								
6 Dt	S,F	7-28-75	10	19	7.2	7.5	19	6.0	8.0	8.5	0(24)
6 Dt	S,F	7-29-75	1	19	6.8	8.0	19	6.0	8.0	>96	6(120)

Species:
*Dm = Daphnia magna
Dt = Dugesia tigrina
Ht = Helisoma trivolvis
Pp = Pimephales promelas

LC_{50}: 3.2 mg/l

**S = static, D = dynamic, F = fluorescent
X = Xenon, SL = solid, LI = liquid

Remarks:

12-4-75/bdo

AQUATIC TOXICITY REPORT - E.K. CO. - HEALTH AND SAFETY LABORATORY

Chemical: CD-3

Source: Tom Dagon

Animals* No. and Species	Cond.**	Date	Dose Range mg or µl/l	Test Start Temp.	Test Start DO	Test Start pH	Test End Temp.	Test End DO	Test End pH	LT50 hours	Survivors at test end (hours)
6 Ph	SL S,F	11-25-74	100 mg/l	17	9.2	6.4	18	5.4	6.4	1.4	0(4)
6 Ph	S,F	2-23-75	10 mg/l	17	7.1	8.3	19	6.5	8.1	34	0(48)
6 Ph	S,F	3-3-75	1 mg/l	21	7.8	8.0	19	7.8	8.0	>96	6(96) LC_{50} = 3.2 mg/l
6 Pl	S,F	11-25-74	100 mg/l	17	9.2	6.4	18	5.4	6.4	1.4	0(4)
6 Pl	S,F	2-23-75	10 mg/l	17	7.1	8.3	19	6.1	8.0	60	0(72)
6 Pl	S,F	3-3-75	1 mg/l	21	7.8	8.0	19	7.8	8.0	>96	6(96) LC_{50} = 3.2 mg/l

HSL No. 74-243

*Pp = Pimephales promelas **S = static D = dynamic X = Xenon

Dm = Daphnia magna F = fluorescent SL = solid LI = liquid

Ph = Physa species Pl = Planorbis species

3-25-75/bdo

Remarks:

OCT 20 1983

905401

BC-83-EN45 20

180178S

ENVIRONMENTAL TEST REPORT

BASIC ENVIRONMENTAL PROFILE FOR:

N-(2((4-Amino-3-methylphenyl)ethylamino)ethyl)methanesulfonamide Sulfate (2:3) Monohydrate (CD-3)

Accession No.: 905401 HS/HF Laboratory Nos.: 74-0243/82-0239

CAS Reg. No.: 24567-76-8

Prepared By:

Rodney J. Boatman, Ph.D.

Biochemistry Section

Health, Safety, and Human Factors Laboratory

August 17, 1983

HEALTH, SAFETY AND HUMAN FACTORS LABORATORY
EASTMAN KODAK COMPANY, Rochester, N.Y. 14650

NOT FOR PUBLICATION

August 17, 1983

SUMMARY OF ENVIRONMENTAL TESTING

Accession No.: 905401 HS/HF Laboratory Nos.: 74-0243/82-0239

CAS Reg. No.: 24567-76-8

Compound Name: N-(2-((4-Amino-3-methylphenyl)ethylamino)ethyl)-methanesulfonamide Sulfate (2:3) Monohydrate (CD-3)

Tests Completed

The following tests have been run on this compound:

- Physical/Chemical Properties
- Oxygen Demand (COD, TOD, BOD_5)
- Environmental Degradation
  - Aerobic Biological Degradation
  - Anaerobic Biological Degradation
  - Oxidative Degradation
- Acute Aquatic Effects
  - Static (seven species)
  - Flow-Through (one species)
- Secondary Waste Treatment Compatibility

Accession No.: 905401 HS/HFL Nos.: 74-0243/82-0239

Octanol/Water Partition Coefficient

Plant Growth Effects

Germination (three species)

Seedling Growth (four species)

Algal Growth (one species)

Physical/Chemical Properties

The melting point of CD-3 was measured using differential scanning calorimetry (DSC) and was found to be 123-124°C. The exo-endothermic profile of CD-3 between 70° and 340°C is shown in Figure 1. A combination of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) was used to determine the thermal stability of the test article in air and nitrogen. The DSC curve in air (Figure 1) indicates a premelt endotherm with an onset of 106-108°C (extrapolated). Melting occurred at 123-124°C. The DSC data for both air and nitrogen atmospheres are similar which would indicate that the degradation process is not limited to oxidation. Thermogravimetric analysis (Figure 2) indicates a 4.4 to 4.6% weight loss over the temperature range 90-225°C. This may be associated with the loss of one equivalent of hydrated water (theoretical weight loss of 4.1%). Bulk weight loss occurs above 225°C.

Accession No.: 905401 HS/HFL Nos.: 74-0243/82-0239

A shake-flask method[1] was used to determine the limiting solubility of the test chemical in distilled water. Test solutions were prepared in deoxygenated distilled water by adding 40, 50, and 60 grams of the test chemical to duplicate, 125 mL glass stoppered Erlenmeyer flasks containing 100 mL of water. Flasks were then sealed with Parafilm™ to reduce uptake of atmospheric oxygen. All flasks were shaken at 100 revolutions/minute for 24 hours at 25°C. After which, the contents of each flask was filtered using a glass-fiber filter system. The concentration of CD-3 in each filtrate was determined by U.V. spectrophotometry. The limiting aqueous solubility is 380 ± 24 g/L (± 1 s.d.). At pH 10.0, CD-3 is reported to have a limiting solubility of 18 g/L.[5]

The dissociation constants for the test chemical were determined using a titration procedure.[2] A 0.01 M aqueous solution of the test chemical was titrated with 0.1 N sodium hydroxide. Two pK values were determined (see Figure 3): $pK_1 = 2.64$; $pK_2 = 6.49$.

The ultraviolet absorption spectrum of CD-3 was measured at three pH values. All test solutions were sparged with nitrogen gas to reduce dissolved oxygen to below 1.5 mg/L. Absorption maxima and

molar absorption coefficients determined at each of the three pH values are: pH 2.0, 1.89×10^3 at 239 nm; pH 6.5, 1.06×10^4 at 239 nm; pH 11.7, 1.34×10^4 at 253 nm.

Oxygen Demand

The TOD value for this compound has been reported as 1.30 g/g[3] and 1.58 g/g.[4,5] The COD value has been reported as 0.90 g/g[3,4] and 1.14 g/g.[5] The BOD_5 value for this compound has been reported as 0.095 g/g[4] and 0.1 g/g.[3] Using an acclimated sludge inoculum the BOD_5 value was 0.391 g/g.[5] Chemical oxidation with pure oxygen consumes 0.176 g of oxygen per gram of test article.[5] Correction of the latter (acclimated) BOD_5 value for chemical degradation gives 0.215 g/g or a BOD_5/COD ratio of 0.24.

Environmental Degradation

The potential for CD-3 to biologically degrade under aerobic conditions was studied in a test designed to measure ready biodegradability.[6] The test involved determination of carbon dioxide evolution, dissolved organic carbon loss, and loss of parent chemical from buffered test solutions containing a dilute (1% by volume) microbial inoculum. During the 21-day test, less than 7% of the theoretical maximum of carbon dioxide was evolved. No significant loss of dissolved organic

carbon was observed. Complete primary degradation of CD-3 from both test and compound control (without inoculum) solutions was observed by day 14 of the test.

A procedure recommended by the American Society for Testing and Materials[7] (ASTM) was used to determine the potential of CD-3 to degrade in an anaerobic environment. A 50 ppm (as carbon) solution of CD-3 was sealed in oxygen-free serum bottles containing an anaerobic medium and 10% (v/v) primary digester sludge.[8] Any methane and carbon dioxide produced in the test bottles was measured with a calibrated pressure transducer and compared to gas evolution in appropriate control bottles. The results are plotted in Figure 4. The negative gas production values recorded for the test article suggest that CD-3 at 50 ppm may have been inhibitory to the anaerobic sludge organisms. Lower test article concentrations were not feasible using this test procedure.

The rate of oxidation of CD-3 in water was determined by monitoring its concentration in aerated solutions over a two day period.[9] Test solutions containing 50 and 500 mg/L of CD-3 were adjusted to pH 7.0 $\pm$ 0.1 and then aerated at 0.2 ft^3/hour. A dissolved

oxygen level of greater than 5.8 mg/L was maintained throughout the test. A control solution containing 500 mg/L of test article under a nitrogen sparge of 0.2 ft^3/hour was monitored as a control. The dissolved oxygen level of the control remained below 1.2 mg/L throughout the test. The results from this study are plotted in Figure 5. The oxidation rate constant calculated using data for the first 12 hours of the test from both test solutions (50 and 500 mg/L) was -0.0685 hr^{-1}. This is equivalent to a half-life of 10.5 hours.

Acute Aquatic Effects

The effects of CD-3 on seven species of aquatic organisms was determined in a static, acute aquatic effects test. All species were exposed to 20 L of test solution at nominal concentrations of 100, 10, 1.0 and 0.1 mg/L. Glass cuboidal containers were used to contain the test solutions and larger aquatic organisms (fish and snails). Small aquatic organisms (water fleas, flatworms, segmented worms, sideswimmers, and pill bugs) were held in stainless steel wire baskets suspended in the test solution. Complete mortality of all species was observed at 100 mg/L. At 10 mg/L, complete mortality of fathead minnows, water fleas, flatworms, and

segmented worms was observed; 20% mortality of sideswimmers was observed. This same concentration caused stress in snails; no adverse effects were noted for pill bugs. A 1.0 mg/L solution caused 20% mortality of both sideswimmers and fathead minnows with no significant effects noted in other species. There were no adverse effects on any of the seven aquatic species at 0.1 mg/L. The LC_{50} ranges determined from this test are: fathead minnows, 1-10 mg/L; water fleas, 1-10 mg/L; sideswimmers, 10-100 mg/L; flatworms, 1-10 mg/L; snails, 10-100 mg/L; segmented worms, 1-10 mg/L; and pill bugs 10-100 mg/L.

A flow-through, aquatic effects test was performed with fathead minnows. A 500 mg/L stock solution of CD-3 was prepared by dissolving 4.5 grams of CD-3 in 9.0 L of nitrogen purged diluent water. This stock solution received a continuous nitrogen purge and was used to obtain upon dilution test solutions with nominal concentrations of 5, 10, and 15 mg/L. Each aquarium received test solution at 100 mL/minute giving 7.2 changes per day of the 20 L test volume. The concentration of CD-3 in each aquarium was measured daily. Mean measured exposure concentrations ($\pm$ 1 s.d.) were 8.39 $\pm$ 2.11, 5.72 $\pm$ 1.04, and 1.95 $\pm$ 1.25 mg/L. Based on

the measured concentrations of CD-3 in the test aquaria, a dynamic LC_{50} value of 3.6 mg/L was determined for fathead minnows.

Secondary Waste Treatment Compatibility

It has been reported that CD-3 is inhibitory to secondary waste treatment microorganisms at a concentration of 100 mg/L.[5] This result was determined using bench-scale, continuous-flow activated sludge units. In this study, duplicate units were fed a synthetic sewage feed. Included in the feed to one unit were various concentrations of color developer. Comparisons of effluent BOD and COD were used to monitor for inhibition.

A similar inhibitory concentration was observed in a radiorespirometric assay procedure. At 150 mg/L, CD-3 decreased by 50% the metabolism of C-14 labelled glucose by sludge microorganisms during a 5-hour incubation.

Octanol/Water Partition Coefficient

The octanol/water partition coefficient was estimated using reverse phase liquid chromatography comparing the retention time of CD-3 to the retention times of chemicals with known P_{ow} values.[10] By this procedure, the log P_{ow} for CD-3 at pH 7.5 was determined to be 0.67. The following chemicals were used as standards in

this determination (Figure 6): p-aminophenol (log P_{ow} = 0.04); acetanilide (log P_{ow} = 1.16); aniline (log P_{ow} = 0.90); acetophenone (log P_{ow} = 1.66); anisole (log P_{ow} = 2.11); benzonitrile (log P_{ow} = 1.56); toluene (log P_{ow} = 2.69); and o-dichlorobenzene (log P_{ow} = 3.38).

Plant Effects

The effects of CD-3 on terrestrial plants were determined using two test procedures. A seven-day germination test was performed with ryegrass, radish, and lettuce seeds. Seeds were germinated in plastic pouches containing distilled water solutions of CD-3. No effect was observed on the germination of ryegrass at 100 mg/L, however, the roots of radish and lettuce were inhibited at this concentration. The no-effect concentration for radish and lettuce was 10 mg/L. Effects of CD-3 on the growth of radish, lettuce, marigold, and corn seedlings were determined. Two week old seedlings were sub-irrigated with tap water containing CD-3. No effect on seedling growth was observed for any of the species tested at a concentration of 100 mg/L.

The toxicity of the test chemical to the algal species, Selenastrum capricornutum, was determined in a 7-day bioassay procedure.[5] Cell growth was measured by monitoring the visible absorption of

cultures of 750 nm. On the final day of testing, measurements of dry weight and chlorophyll-a were obtained. Growth rates (based on absorbance values) and 7-day cell yields were used to assay for cell growth inhibition. Inhibition was seen at 4.0 mg/L but not a 0.4 mg/L.

Conclusions

The test article was acutely toxic at 100 mg/L to all seven species of aquatic organisms tested. The most sensitive aquatic species were the fathead minnows, water fleas, and pill bugs, with LC_{50} ranges of 1-10 mg/L. CD-3 may inhibit the early growth of some terrestrial plant species at concentrations >10 mg/L. Inhibition of algal growth may occur above 0.4 mg/L. Biological degradation of CD-3 does not readily occur under either aerobic or anaerobic conditions. However, it has a low potential to bioconcentrate. The oxidative half-life in water of 10.5 hours would suggest that the test chemical will not persist under aerobic conditions within the aquatic environment. Thus, chronic exposures are unlikely.

Based on the results of this study, single or intermittent exposures to concentrations of CD-3 $\leq$0.1 mg/L are not expected to have adverse environmental effects.

Accession No.: 905401 HS/HF Laboratory Nos.: 74-0243/82-0239

References

1. "Water Solubility (Flask Method)," Method 105, OECD Guideline for Testing of Chemicals, (1981).

2. "Dissociation Constants in Water (Titration Method)," Method 112, OECD Guideline for Testing of Chemicals, (1981).

3. Deegan, R.O., Jr. and Petschke, D.C., "A Biological and Chemical Characterization of Photoprocessing Chemicals," Photographic Technology Division, Technical Report A-550, June 3, 1977.

4. Beach, M.W., "Toxicity and Biodegradation of Processing Chemicals: First Revision," Photographic Technology Division, Technical Report A-534, June 21, 1973.

5. National Association of Photographic Manufacturers, Inc. and Hydroscience, Inc., "Environmental Effects of Photoprocessing Chemicals," National Association of Photographic Manufactures, Harrison, New York, 1974, 2 volumes.

6. OECD Chemical Testing Program, Report of Expert Group, Degradation/Accumulation, Final Report, Test Guidelines C104/79 (1979).

Accession No.: 905401 HS/HF Laboratory Nos.: 74-0243/82-0239

7. "Proposed Standard Practice for the Determination of the Anaerobic Biodegradability of Organic Chemicals," American Society for Testing and Materials, Draft No.5, E57.06, May, 1982.

8. Sludge collected from the primary, anaerobic digester of the Waste Water Treatment Plant, W. Main Street, Avon, New York.

9. "Screening Test for Oxidation of ArOH, ArN(H)R or RSH by $RO_2^{\bullet}$," Method 4.5.2, Laboratory Protocols for Evaluation the Fate of Organic Chemicals in Air and Water, EPA-600/3-82-022, p. 110, (1982).

10. Moszkowicz, V.O., "Determination of Partition Coefficients by Liquid Chromatography," Technical Memorandum, No. 81-09, July 7, 1981.

Figure 1.

Differential Scanning Calorimetry

Results for CD-3

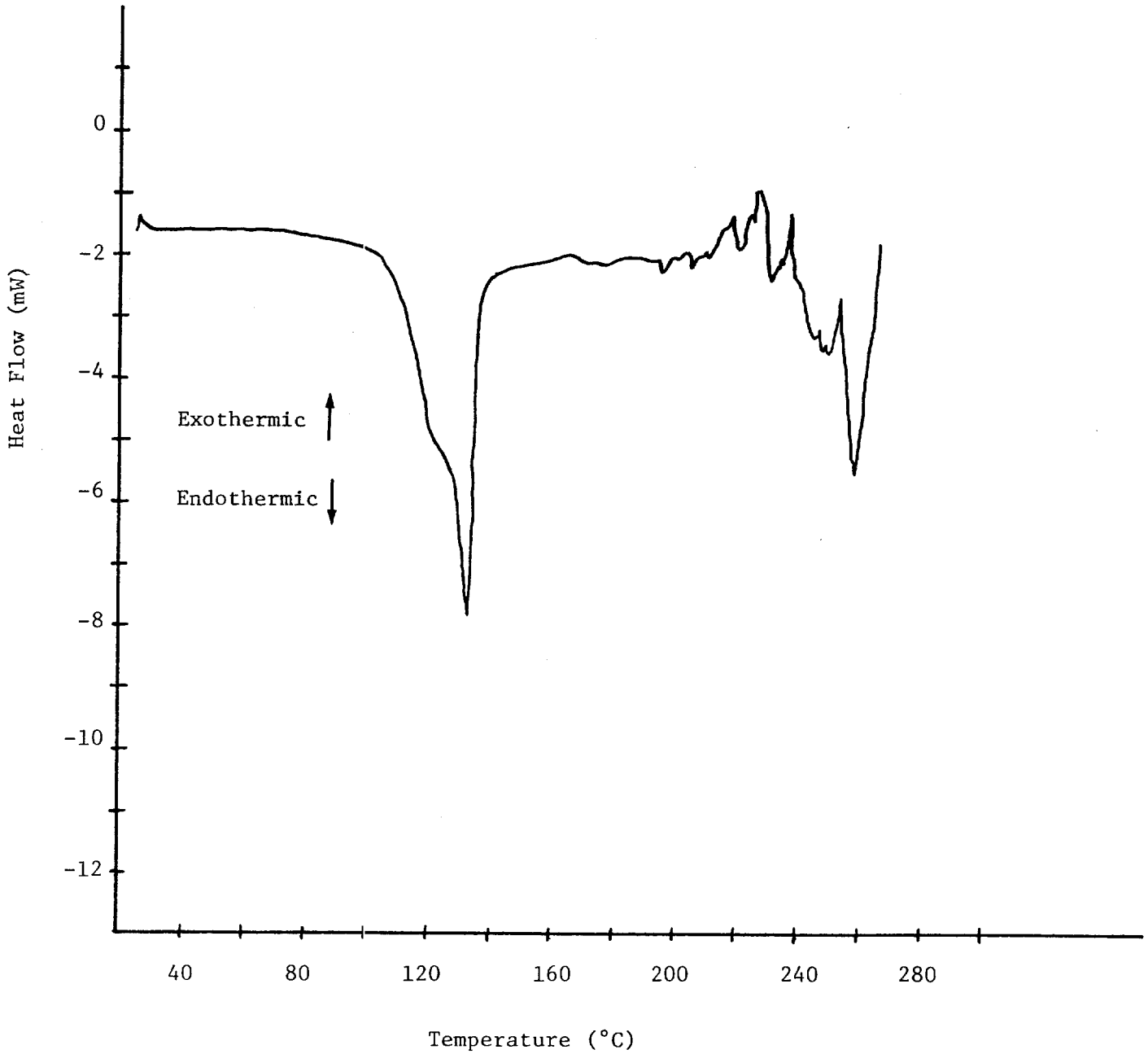


Figure 2.

Thermogravimetric Analysis

Results for CD-3

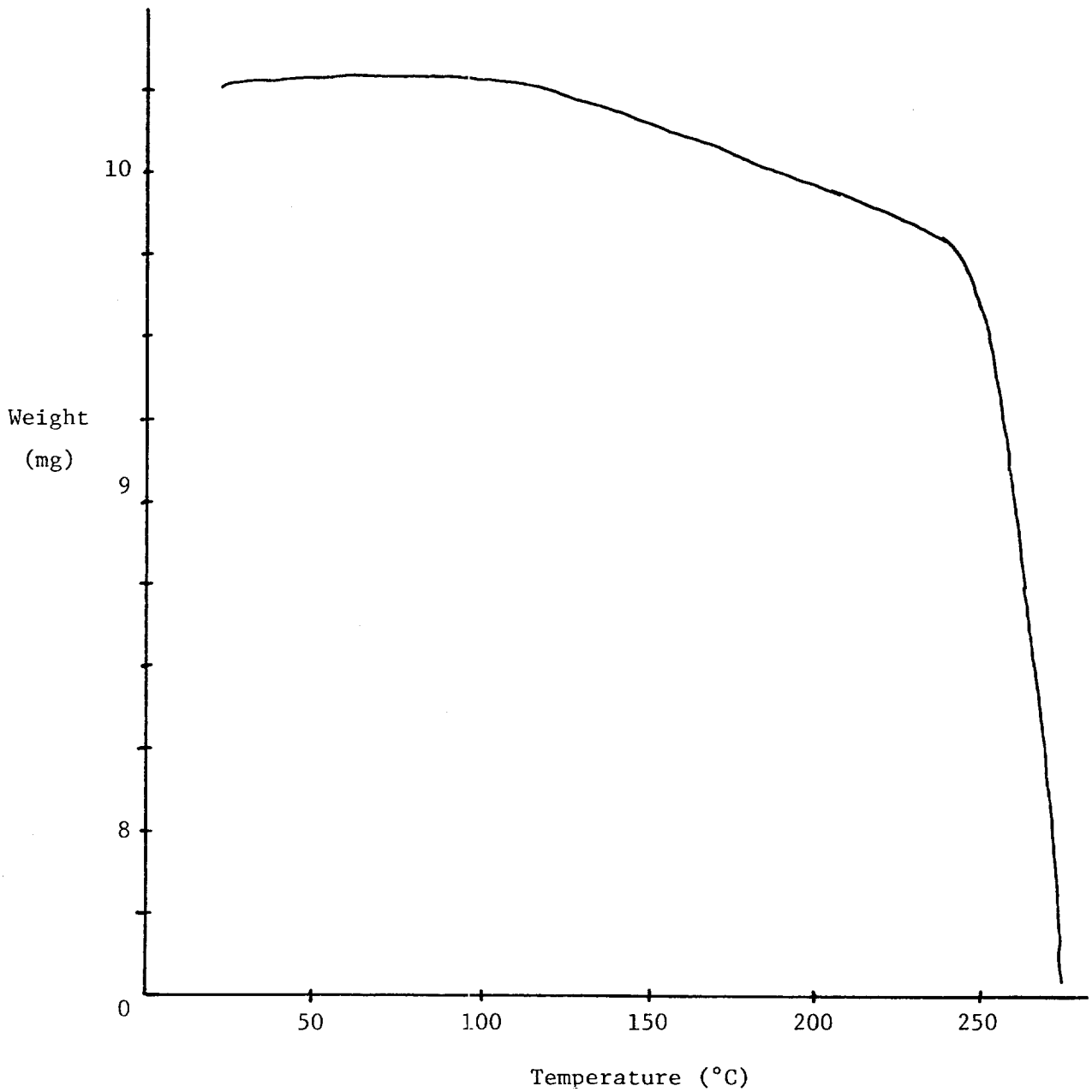


Figure 3.

Dissociation Constant Data

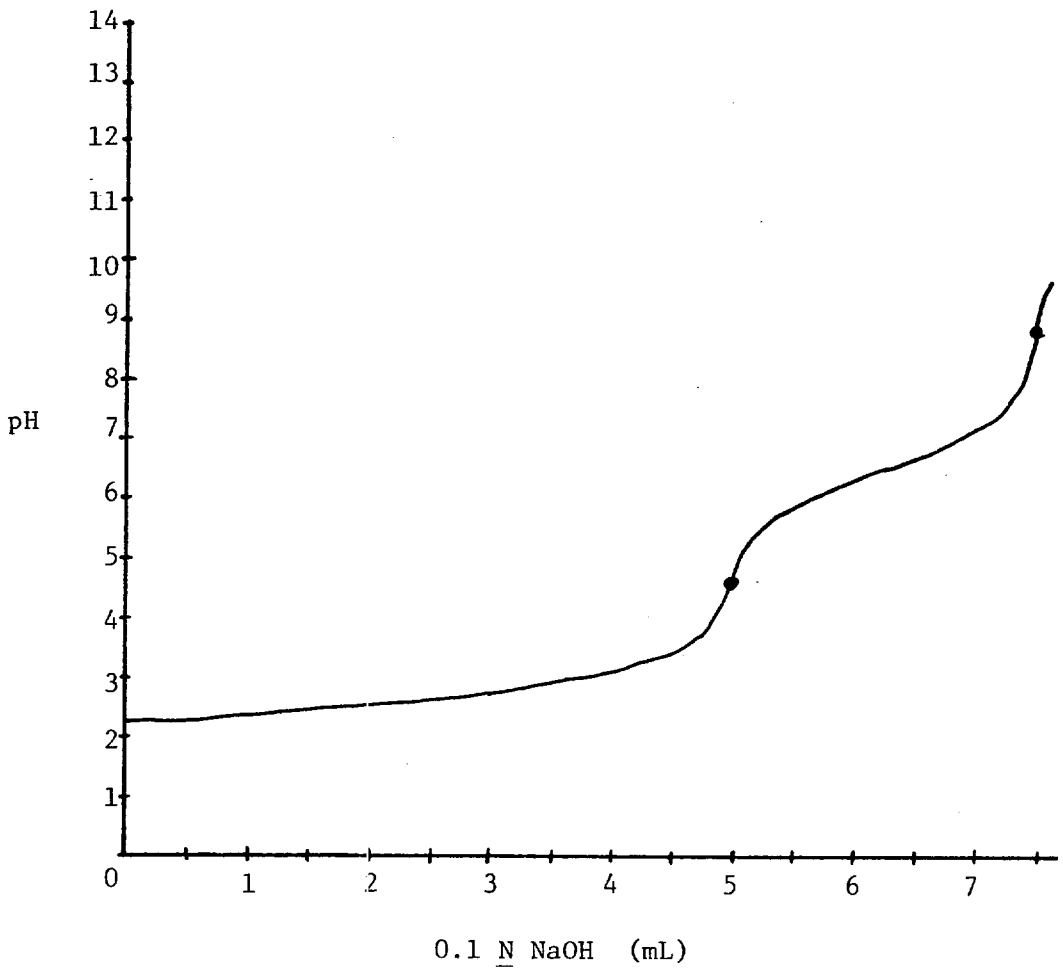


Figure 4.

Anaerobic Biodegradation of CD-3

Cumulative CO_2 and CH_4 Production

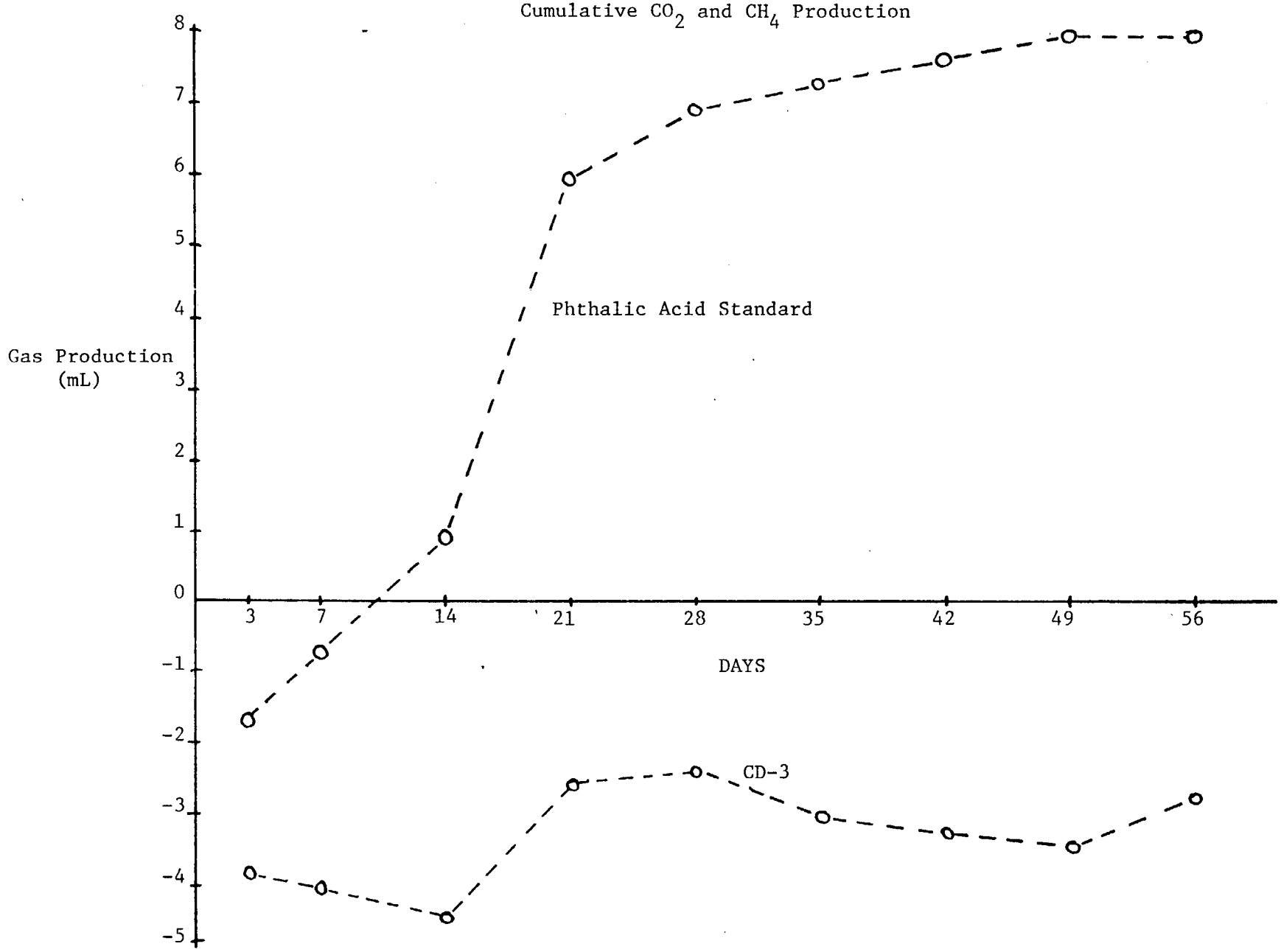


Figure 5.

Oxidation of CD-3 in Water

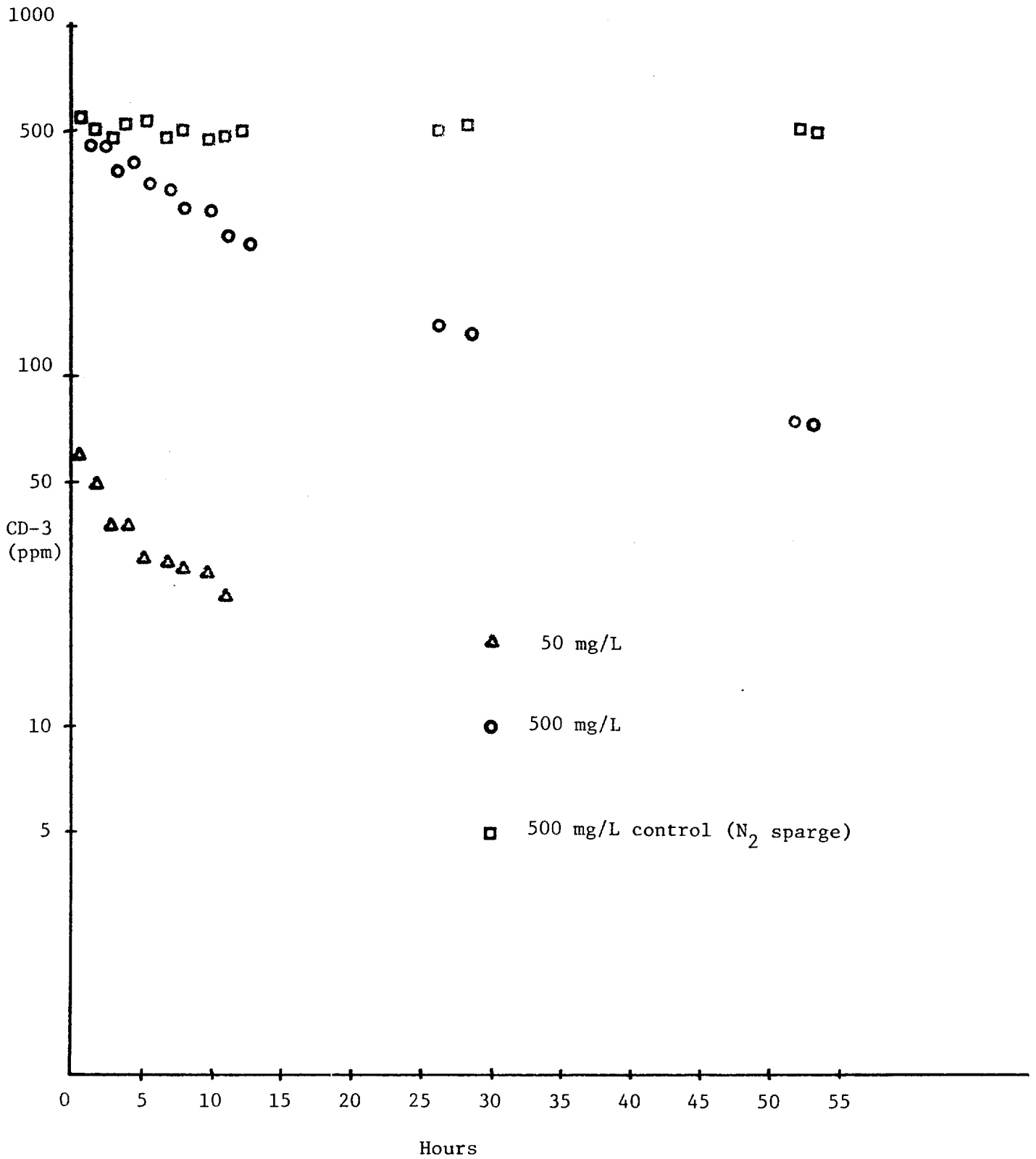
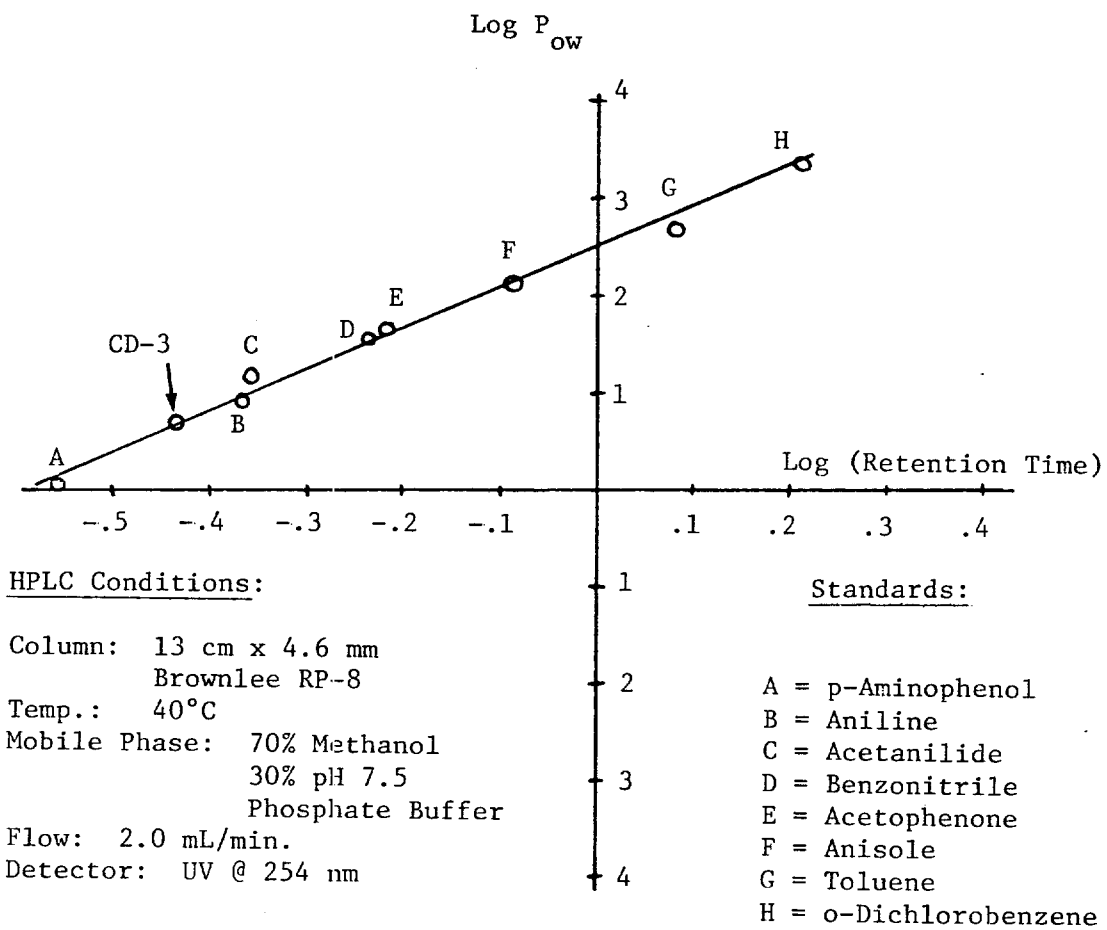


Figure 6.

Partition Coefficient Data

For CD-3



Prepared by Rodney J. Boatman
Rodney J. Boatman, Ph.D.

Approved by Kenneth A. Robillard
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Approved by B. D. Astill 9/21/83
Bernard D. Astill, Ph.D.
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Biochemistry Section

ENVIRONMENTAL SAFETY DATA SHEET

HEALTH SAFETY AND HUMAN FACTORS LABORATORY

ENVIRONMENTAL SCIENCES GROUP

QUESTIONS CONCERNING DATA ON THIS SHEET SHOULD BE REFERRED TO THE ENVIRONMENTAL SCIENCES GROUP, EXTENSION: 85211

CAS REG. NO.	HSHFL NO.	MOLECULAR WT.	DATE	ACCESSION NO.
24567-76-8 (1)	74-0243	436.5	May 11, 1982	905401

EMPIRICAL FORMULA	SRID #
$C_{12}H_{21}N_3O_2S \cdot 3/2\ H_2SO_4 \cdot H_2O$	

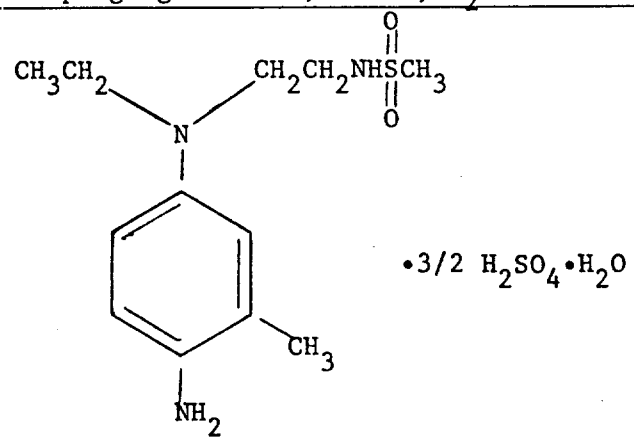
COMPOUND NAME

N-{2-[(4-Amino-3-methylphenyl)ethylamine]ethyl}methanesulfonamide Sulfate (2:3) Dihydrate

SYNONYMS

CD-3; Color Developing Agent CD-3; D109B; D_2W

STRUCTURE



TSCA INVENTORY LIST	REGULATORY STATUS
☒ YES ☐ NO	CD-3 Base(1) is listed in the Reg. of Toxic Effects of Chem. Substances(2)

PHYSICAL CHEMICAL PROPERTIES

MELTING POINT	123-127°C(3); 168-169°C(4)
BOILING POINT	
VAPOR PRESSURE	mm@ °C
PH of 0.1% AQUEOUS SOLUTION	
PKa	Ka
OTHER	
REMARKS	

SOLUBILITY

H_2O	18 g/L at pH 10.0(3)
OCTANOL	
CORN OIL	
ACETONE	
DMSO	
OTHER	

OXYGEN DEMAND TESTS

TOD	COD	BOD_5	BOD_{20}
1.58 g/g(5); (6) 1.30g/g(7)	0.90g/g(5); (7) 1.14g/g(6)	.095g/g(5); 0.1g/g(7)	

REMARKS The BOD_5 reported for an acclimated sludge is 0.391 g/g.(6) Chemical oxidation with pure oxygen required 0.176 g of oxygen/g of CD-3. The BOD_5 measurements, therefore, represent simple oxygen demand rather than any biological degradation(6).

ENVIRONMENTAL HALF-LIFE

T ½ BIOLOGICAL	
PRODUCTS OF BIODEGRADATION OR BIOTRANSFORMATION	
T ½ PHOTOCHEMICAL	(fc@ nm)
PRODUCTS OF PHOTODEGRADATION	
T ½ HYDROLYSIS	
PRODUCTS OF HYDROLYSIS	

REMARKS At pH >5, this compound is expected to readily degrade via several possible oxidative pathways. Oligomers and humic-type compounds are some of the degradation products.

HSHFL NO.	ENVIRONMENTAL SAFETY DATA SHEET HEALTH SAFETY AND HUMAN FACTORS LABORATORY ENVIRONMENTAL SCIENCES GROUP	ACCESSION NO.
74-0243		905401

ACUTE AQUATIC EFFECTS

LC_{50} - PIMEPHALES PROMELAS (FATHEAD MINNOW)	6.4 mg/L (F) (6)	LC_{50} - HELISOMA TRIVOLVIS (SNAIL)	3.2 mg/L* (F)
LC_{50} - DAPHNIA MAGNA (WATER FLEA)	0.3 mg/L (F) (6)	LC_{50} - ASELLUS INTERMEDIUS (PILL BUG)	
LC_{50} - GAMMARUS FASCIATUS (SIDESWIMMER)		LC_{50} - LUMBRICULUS VARIEGATUS (SEGMENTED WORM)	
LC_{50} - DUGESIA TIGRINA (FLATWORM)	3.2 mg/L*(F)(120hr)	Physa sp.	3.2 mg/L*(F)

REMARKS X= Xenon Lighting. F= Fluorescent Lighting. *Test article 74-0243. [Pp: 96-hr bioassays, X, LC_{50}= 12.8 mg/L (1967), 16.0 mg/L (1970)]. [Dm: 96-hr, renewal, F, LC_{50}= 3.2 mg/L; 3-week bioassay, F, LC_{50}= 1.0 mg/L (1973)].

SECONDARY WASTE TREATMENT COMPATIBILITY

IC_{50} 150 mg/L

REMARKS The test article was shown to be inhibitory to conventional waste treatment systems at a level of 100 mg/L.(6)

BIO-CONCENTRATION POTENTIAL

WLN	OCTANOL/H_2O COEFF.	T ½ - CLEARANCE

BIOCONCENTRATION FACTOR

REMARKS

PLANT GROWTH EFFECTS NO EFFECT CONCENTRATION

GERMINATION		SEEDLING	
LOLIUM PERENNE (RYEGRASS)	100 mg/L	ZEA MAYS (CORN)	100 mg/L
RAPHANUS SATIVUS (RADISH)	10 mg/L	TAGETES PATULA (MARIGOLD)	100 mg/L
LACTUCA SATIVA (LETTUCE)	10 mg/L	LACTUCA SATIVA (LETTUCE)	100 mg/L
OTHER		RAPHANUS SATIVUS (RADISH)	100 mg/L

REMARKS

The green alga, Selenastrum capricornutum was inhibited by 4.0 mg/L of the test article, but not by 0.4 mg/L.(6)

REFERENCES /COMMENTS

This compound is also known by the following names:

N-[2-(4-Amino-N-ethyl-m-toluidino)ethyl]methanesulfonamide Sesquisulfate Monohydrate

4-Amino-N-ethyl-N-(beta-methanesulfonamidoethyl)-m-toluidine Sesquisulfate Monohydrate

4-Amino-3-methyl-N-ethyl-N-(beta-sulfonamidoethyl)aniline Sesquisulfate Monohydrate

References are on a separate page.

ENVIRONMENTAL SAFETY EVALUATION

Tier Testing Ratings

(Environmental Risk Analysis for Chemicals, "Sequential Testing for Chemical Risk Assessment", in publication)

E = Estimated	LOW (1)	MED (2)	HIGH (3)
PERSISTENCE	1E		
SECONDARY WASTE TREATMENT EFFECTS			3
ACUTE AQUATIC EFFECTS			3
BIOCONCENTRATION	1E		

PREPARED BY: Philip W. Jenkins/Joseph W. Gorsuch

DATE: May 11, 1982

KPD-0051-910 (8/81)

References for CD-3 Acc. No. 905401

1. A second CAS Registry Number [59211-82-4] was also assigned to this compound. This second number is nct presently valid and will not be used in future CAS products and services. However, both of these numbers and all numbers mentioned below should be used in any comprehensive retrospective search. CD-3 free base is Acc. No. 905400; CAS Reg. No. 92-09-1. Other salts listed in Kodak files are: dihydrochloride, Acc. No. 195151, CAS Reg. No. 56773-57-0; monosulfate, Acc. No. 113439, CAS Reg. No. 20191-53-1 [the "sulfate" has CAS Reg. No. 7207-43-4]; di-p-toluenesulfonate, Acc. No. 149744, CAS Reg. No. _____; hemisulfate, Acc. No. 219790, CAS Reg. No. _____; and monophosphate, Acc. No. 344543, CAS Reg. No. 56046-61-8 [the "phosphate" has CAS Reg. No. 56773-58-1]. Registry numbers of other salts are available from the chem. Abstr. molecular formula index and from on-line data bases.

2. Fairchild, E.J., Ed., "Registry of toxic Effects of Chemical Substances--1978 Edition, "National Institute for Occupational Safety and Health, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio, October, 1978.

3. Bent, R.L., Private Communication, May 1, 1978.

4. Bent, R.L., Dessloch, J.C., Duennebier, F.C., Fassett, D.W., Glass, D.B., James, T.H., Julian, D.B., Ruby, W.R., Snell, J.M., Sterner, J.H., Thirtle, J.R., Vittum, P.W., and Weissberger, A., "Chemical Constitution, Electrochemical, Photographic and Allergenic Properties of p-Amino-N-dialkylanilines," J. Amer. Chem. Soc., 73, 3100-3125(1951).

5. Beach, M.W., "Toxicity and Biodegradation of Processing Chemicals: First Revision," Photographic Technology Division Technical Report A-534, June 21, 1973.

Acc. No. 905401.

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A Comparative Study of the Acute Aquatic Effects of CD-3 Under Laboratory and Artificial Sunlight Conditions

Accession No. 905401 HAEL No. 82-0239

By

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Accession No. 905401 HAEL No. 82-0239

Abstract

The acute toxicity of the color developing agent N-(2-[(4-amino-3-methylphenyl)ethylamino]ethyl)methanesulfonamide Sulfate (2:3) Monohydrate - Color Developer No.3 - (CD-3) to the fathead minnow (Pimephales promelas) has been established at 2-4 mg/L. This high potential to affect aquatic vertebrates was similarly replicated during toxicity tests using several invertebrate species.

In order to better understand the degradation of CD-3 under natural environmental conditions, a series of time-weighted static acute aquatic toxicity tests with CD-3 was made using fathead minnows as the test species. The artificial sunlight photolysis of CD-3 was compared to its degradation under fluorescent lighting. Solutions initially containing 50 mg/L of CD-3 were exposed to either fluorescent light (100 ft-candles) or xenon arc light (6000 ft-candles). Degradation of the CD-3 solutions was monitored by high performance liquid chromatography and aqueous exclusion chromatography. The toxicity to fathead minnows of the solutions after selected periods of illumination was also determined. The chemical analyses showed that all of the measurable CD-3 photodegraded under xenon arc lighting (artificial sunlight) within 24 hours with some higher molecular weight degradation products being formed. After 72 hours of artificial sunlight illumination the 50 mg/L solutions were no longer toxic to the minnows. The rate of CD-3 degradation was three times faster under artificial sunlight than under fluorescent lighting.

The results of this test indicate that CD-3 will photodegrade in the aquatic environment, and this process results in removal of acute aquatic toxicity.

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TESTING FACILITY

Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14650
USA

DATE OF STUDY INITIATION April 18, 1983

DATE OF STUDY COMPLETION July 23, 1987

STUDY PARTICIPANTS

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PURPOSE/OBJECTIVE

During a comprehensive study of the color developer CD-3, chemical name N-(2-[(4-amino-3-methylphenyl)ethylamine]ethyl)methanesulfonamide Sulfate (2:3) Monohydrate, by the Environmental Sciences Section, it was observed that CD-3 has a high potential to acutely affect aquatic organisms. The LC_{50} values for several aquatic species were in the range of 1-10 mg/L.

This study was designed to compare the acute aquatic effects of aerated CD-3 solutions on Pimephales promelas (fathead minnows) under two different lighting conditions: cool white fluorescent (laboratory illumination) and xenon arc (artificial sunlight illumination). The fathead minnow, Pimephales promelas, was chosen for this test based on its relative sensitivity as a representative freshwater species of fish. In this test, the fathead minnows were observed for signs of stress, as well as mortality, when exposed to the test article. The information from this test can be used to determine the likelihood of an effect if the test article enters the freshwater environment.

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TEST ARTICLE

Identity: Color Developer Three (CD-3)

Sample Laboratory No.: 82-0239

CAS Registration No.: 25646-71-3

Physical/Chemical Properties[1]

Purity: Not determined

Solubility: 380±24 g/L (± 1 S.D.) in water

MATERIALS AND METHODS

Test System

Phylum - Chordata

Class - Osteichthyes

Order - Cypriniformes

Family - Cyprinidae

Genus - *Pimephales*

Species - *promelas* (Rafinesque)

Source of Fathead Minnows

Juvenile fathead minnows were obtained from Sea Plantations, Inc., Salem, MA 01970, and held in the Eco-Chem Testing Group animal colony room for at least two weeks prior to use. This particular batch of fish was received by the Eco-Chem Testing Group on March 16, 1983.

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Source of Dilution Water

The water used in this test was pumped from Lake Ontario by the Kodak Park Lake Station Water Treatment Facility into a large underground storage reservoir located near the Environmental Sciences Section testing facility (Building 306, Kodak Park). This water subsequently was pumped into the laboratory where it passed through 3 µm polypropylene filter tubes, then through a series of powdered, activated carbon filter tubes, and then through a final set of 3 µm polypropylene filter tubes. Prior to passing through a tempering heat-exchange unit, the filtered diluent water stream received 150 ppb of $Na_2S_2O_3$ via a chemical injection system. This treatment further reduces trace levels of residual chlorine. The filtered-treated-tempered water was then distributed throughout the laboratory through a stainless steel piping system. Upon reaching the testing laboratory, the filtered-treated-tempered water was cascaded through a column degassing unit and held in an open aeration basin prior to use. Test diluent water was drawn from this source. Representative values for the hardness (as $CaCO_3$) and the total alkalinity (as $CaCO_3$) of the diluent water for the study period were 130 mg/L and 94 mg/L, respectively (Attachment 2, Summary of Chemical Characterization of Dilution Water).

Acclimation Procedure

All test organisms were acclimated to the diluent water prior to the test since the same filtered-treated-tempered water and filtered, compressed air used for all laboratory water/aeration processes during the test were supplied continuously to the stainless steel rearing tanks. All aquatic organism populations used in the laboratory are maintained in this water for at least two weeks before being used in a test.

Size And Number of Fathead Minnows Used at Each Concentration

Juvenile fathead minnows, as uniform in size as possible, were collected from the holding tanks. Sequential randomization was accomplished by allocating no more than 20% of any one set of test organisms at a time to each vessel used for collection. Biological loading within test vessels was kept below 0.5 g wet weight per liter of test solution. The average wet weight for one of the twelve sets of minnows (10 minnows/set) was determined at the start of the test. These fathead minnows were subsequently used as one of the control sets of test organisms.

Preparation Of the Test Solutions

The test article was introduced as a solid with vigorous stirring into each of the eight replicate test vessels at a nominal concentration of 50.0 mg/L. The volume of diluent water in each test vessel was 20 L.

Apparatus and Test Conditions

This acute aquatic effects bioassay was performed in seamless glass 30.5 cm cuboidal Pyrex® chromatography jars containing 20 L of the solution. Each xenon arc-irradiated test vessel received 6000 footcandles measured prior to filling, at the inner surface facing the lamp. These test vessels designated X-1, X-2, X-3, and X-4 were irradiated continuously until the termination of each bioassay.

A set of four control test vessels designated C-1, C-2, C-3, and C-4 were treated similarly to X-1, X-2, X-3, and X-4 in every manner, but received no test article.

A third set of four test vessels were treated similarly to the test and control xenon arc-irradiated sets, but received approximately 60 footcandles of cool white fluorescent irradiation measured at the test article solution-air interface.

All test solutions received 0.05 L per minute aeration supplied through a fine bubble gas dispersion tube. The sintered glass dispersion tip was located 12 to 14 centimeters below the test solution surface.

The test temperature in the xenon arc-irradiated test solutions primarily depended upon the coloration due to degradation of the test article. The fluorescent light-irradiated test solutions were maintained at 20 $\pm$ 1°C.

Test Procedure

The procedures used in this test were based on accepted methodologies.[2-4] Observations for signs of mortality and stress were made during the test at start, 6 hours and thereafter at 24 hour increments. Temperature, dissolved oxygen, and pH were also determined at each observation time and at such time as the entire biological test system was affected.

Ten test organisms were chronologically placed into each of the test and control vessels by the following procedure:

F-1, X-1 and C-1 at 0 hours (test start)
F-2, X-2 and C-2 at 24 hours (after 24 hours of aeration & irradiation)
F-3, X-3 and C-3 at 48 hours (after 48 hours of aeration & irradiation)
F-4, X-4 and C-4 at 72 hours (after 72 hours of aeration & irradiation)

The concentration of the test article in the aerated test and control solutions was measured during the course of the test. To compensate for evaporation prior to sampling for chemical analysis the test solutions were brought back to the 20 L starting volume with distilled water.

Analytical Procedures

A high performance liquid chromatographic (HPLC) procedure for CD-3 was performed on the test solution at: (1) 0 hour (immediately prior to the start of aeration); (2) 2.0, 3.0, 3.7, 4.3, 5.5, 6.2, and 6.8 hours; (3) 24 hours; (4) 48 hours; and (5) 72 hours. This HPLC procedure was performed only on the test article solutions prior to introduction of the minnows. Due to the need for a clean sample matrix no analyses were conducted on a test solution after minnow introduction.

The HPLC parameters were as follows:

Column: Waters µBondapak RP-18, 30 cm x 3.9 mm
Mobile Phase: Isocratic
50% HPLC grade methanol
50% PIC reagent B-6
Flow: 1 mL/minute
Detector: UV, 238 nm @ 0.04 AUFS
Sample Size: 20 µL

Total organic carbon analyses[3] were performed on test solutions designated X-4, F-4, C-4 at times 0, 6, 24, 48, and 72 hours.

An aqueous exclusion chromatographic analytical procedure was used to characterize the soluble components of test solutions X-4, F-4, and C-4 at times 0, 24, 48, and 72 hours. This analysis was used to characterize the soluble degradation products based on molecular weight fractions.

For this aqueous exclusion chromatographic procedure, a 2.5 cm x 22 cm column of Sephadex G-15 (Pharmacia Fine Chemicals) was eluted with 0.01M sodium phosphate (pH 7.0) at a flow rate of 64 mL/hour. A sample size of approximately 1.0 mL, was used for all analyses. Eluted components were measured using a 254 nm UV detector.

Blue Dextran 2000 (Pharmacia Fine Chemicals) was used to determine the column void volume as noted in Figures 3 and 4. Riboflavin (M.W. 376) was used as a low M.W. standard, with retention time shown in Figures 3 and 4.

A colorometric test for tannin-lignins[3] was used to determine the extent to which hydroxylated aromatic compounds were formed upon degradation of CD-3 (see Table 3).

QUALITY ASSURANCE MONITORING

See the attached report of Quality Assurance Monitoring (Attachment 3).

DATA STORAGE AND RECORDS RETENTION

All raw data and summaries of data will be retained for at least ten years by the Health and Environment Laboratories in Building 306 or by the Chemicals Quality Services Division in Building 320 of the Eastman Kodak Company, Kodak Park, Rochester, New York 14650.

RESULTS AND INTERPRETATION

The extremes of temperature (^{o}C) for the test solutions were: F-1/4 (19-20), X-1/4 (19-24) and C-1/4 (19-24). The extremes of pH values were: F-1/4 (7.3-8.0), X-1/4 (7.1-7.9), and C-1/4 (7.8-8.1). The extremes for the dissolved oxygen concentrations (mg/L) were: F-1/4 (7.9-8.6), X-1/4 (7.4-8.6) and C-1/4 (7.9-8.5). These values are considered to be within the tolerance range for the organisms used in this test,[2-6] and are not likely to have caused any adverse effects. The average fish weight determined at test start was 0.28 g.

A temperature increase of 3°C above the 24°C high temperature noted in the C-1/4 system (xenon arc-irradiated controls) was measured in the X-1/4 system (the CD-3 containing test solutions that were simultaneously exposed to the artificial sunlight). This temperature elevation was attributable to increased absorption of incident light by the reddish-purple color present in the 50 mg/L CD-3 test solutions of the X-1/4 system.

A decrease in pH and marked onset of reddish-purple coloration accompanied the rapid degradation in both the fluorescent (0.5 pH unit drop) and xenon arc (0.7 pH unit drop) test solutions during the first six hours.

The resulting data for the HPLC analysis of the test solutions for CD-3 during the initial 72 hours are listed in Table 1. The seven sequential analyses during the first seven hours were limited to test solutions C-4, X-4 and F-4 due to instrumental analysis turn around time. The ln (conc.) time values were fitted to a pseudo first-order rate process. The resulting rate constants are and half-lives are 0.22 hr^{-1} and 3 hrs for X-4 and 0.07 hr^{-1} and 10 hrs for F-4, respectively.

The initial degradation of CD-3 is dominated by its oxidation in aqueous solutions. In a test which measured chemical oxidation, the half-life of CD-3 was determined to be 10.5 hr[1]. The half-life value determined in this test for the F-4 test solution was 10 hr. The closeness of these two half-life values indicates that most, if not all of the degradation of CD-3 in the F-4 solution resulted from chemical oxidation. By comparison illumination with artificial sunlight decreased the half-life of CD-3 by 70%. Thus, in the presence of sunlight, photolysis becomes a significant, if not dominant, factor in the initial degradation of the color developer. These results are in agreement with previously reported data.

The measurements of total organic carbon are presented in Table 2. No significant change in this parameter was detected from the beginning to the end of the study. Although some insoluble material appeared in the test solutions all TOC analysis were performed on unfiltered samples. These data indicate that the color developer did not undergo mineralization under these test conditions. The insoluble material formed upon degradation appears to account for only a small fraction of the CD-3 originally present. Observations and analyses performed during a radiolabelled shake-flask biodegradation test using acclimated microorganisms suggested that some degradation to CO_2 (aerobic) would be observed. A previously conducted anaerobic biodegradation-test indicated degradation to CH_4 (anaerobic) probably would not be observed.

The results for the Tannin-Lignin method for measuring hydroxylated aromatic compounds are listed in Table 3. The data indicate that there was little difference between samples exposed to xenon arc light or fluorescent light.

The aqueous exclusion chromatography results are presented in Figures 3 and 4. The results from the analyses of the F-4 test solutions indicate that a intermediate M.W. fraction (approximate range 376-1500 Daltons) has been formed having an apparent molecular weight greater than that of CD-3. The fraction did not significantly change after 24 hours. CD-3 was detected after 24 hours but had disappeared after 48 hours. Based on manufacturer's data,[5] compounds with apparent molecular weights greater than 1500 Daltons would elute at the void volume. Thus, it appears that the degradation product(s) observed by aqueous exclusion chromatography have as apparent molecular weight greater than CD-3 (436.5) and less than 1500 Daltons.

The results of the analyses of the X-4 test solution indicate at least three fractions of M.W. greater than that of CD-3 have been formed. No CD-3 was detected at 24, 48, or 72 hours. As in the F-4 test solution, no significant amounts of material with an apparent molecular weight greater than 1500 Daltons were seen in any of the analyses.

The percent fish survival for the bioassay systems are listed in Table 4. Total fish mortality was observed by six hours in all fluorescent-exposed test solutions [i.e. there was no increase in the time required to kill 50% of the population at risk (LT_{50})]. Therefore, exposing the CD-3 to fluorescent lighting for 24, 48 and 72 hours irradiation did not alter the effects of the solution on the test organisms.

For the X-1 and X-2 test solutions total mortality also occurred by six hours. For the X-3 test solution (exposed 48 hours to artificial sunlight) the LT_{50} was significantly increased. A previously reported photochemical half-life in distilled water was determined to be 2.5 to 6.2 hours[1]. This time frame falls within the half-life for the X-1/4 artificial sunlight irradiated system. Exposure of the X-1/4 CD-3 test solutions to 72 hours of artificial sunlight resulted in a solution that was no longer toxic to fish.

TEST VALIDITY

The following criteria for a valid test were met during the study.

A. The control mortality was not greater than 10% when adverse effects were noted.

B. The dissolved oxygen level did not fall below 40% of the initial oxygen level when adverse effects were observed in the highest test concentration.

C. No abnormal occurrences (e.g., laboratory accidents) that might have influenced the outcome of the test were noted.

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PROTOCOL DEVIATIONS

None.

CONCLUSIONS

1) Under the conditions of both fluorescent lighting and artificial sunlight, the color developer was observed to degrade rapidly with half-lifes of 10 and 3 hours, respectively.

2) The rate of degradation under conditions of artificial sunlight was faster than under fluorescent lighting. The fluorescent 1/2 life being $\cong$ to hydrolysis 1/2 life.

3) Some higher molecular weight degradation products were formed.

4) The molecular weight of compounds formed under conditions of artificial sunlight are greater than those formed under fluorescent light.

5) The photodegradation of CD-3 resulted in the formation of some higher molecular weight compounds and the eventual reduction in the potential to cause acute aquatic effects.

Accession No. 905401 HAEL No. 82-0239

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5. Pharmacia Fine Chemicals, 1983 Catalog. Table 2. Specifications for gel filtration products. p. 9.

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TABLE 1. High Performance Liquid Chromatography Analyses for the Presence of CD-3

Sampling	Test Solution (mg/L)								
Time (hr.)	C-4	X-1	X-2	X-3	X-4	F-1	F-2	F-3	F-4
0	ND	50.3	50.7	50.4	49.2	47.8	43.4	46.0	43.2
2	ND	NA	NA	NA	41.9	NA	NA	NA	36.0
3	ND	NA	NA	NA	37.4	NA	NA	NA	40.2
3.7	ND	NA	NA	NA	31.2	NA	NA	NA	36.9
4.3	ND	NA	NA	NA	27.3	NA	NA	NA	35.1
5.5	ND	NA	NA	NA	16.3	NA	NA	NA	30.6
6.2	ND	NA	NA	NA	13.3	NA	NA	NA	33.5
6.8	ND	NA	NA	NA	12.9	NA	NA	NA	27.8
24	ND	NA	NQ	NQ	NQ	NA	7.0	6.0	7.4
48	ND	NA	NA	NQ	NQ	NA	NA	NQ	NQ
72	ND	NA	NA	NA	NQ	NA	NA	NA	NQ

ND = none detected (lower limit of detection approximately 0.5 mg/L)

NA = not analyzed due to instrumental analysis turn-around time

NQ = not quantifiable (limit of quantitation = 4 mg/L due to interference from degradation products)

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TABLE 2. Total Organic Carbon Analyses of Test Solutions X-4, F-4, and C-4

Sampling Time (hr.)	Test Solution (mg/L) X-4	F-4	C-4
0	15.32	13.81	1.12
6	13.12	16.51	1.76
24	14.99	13.40	1.69
48	13.76	15.11	1.46
72	13.83	16.16	1.65

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TABLE 3. Tannin-Lignin Analyses for the Presence of Hydroxylated Aromatic Compounds

	Test Solution (mg/L)		
Time (hr.)	C-4 (mg/L)	F-4 (mg/L)	X-4 (mg/L)
0	0	12.3	14.2
6	0	10.6	11.2
24	0	11.7	11.2
48	0	9.5	9.0
72	0	8.4	7.8

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TABLE 4. Percent Fish Survival at 6, 24, and 96-Hours

Test Solution	% Survivors	Time (hr.)
X-1	0	6
X-2	0	6
X-3	20	96
X-4	100	96
F-1	0	6
F-2	0	6
F-3	0	6
F-4	0	6
C-1	100	24
C-2	100	24
C-3	100	96
C-4	100	96

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FIGURE 1. Plot of F-4 and X-4 Concentrations of CD-3 (0-7 hr.)

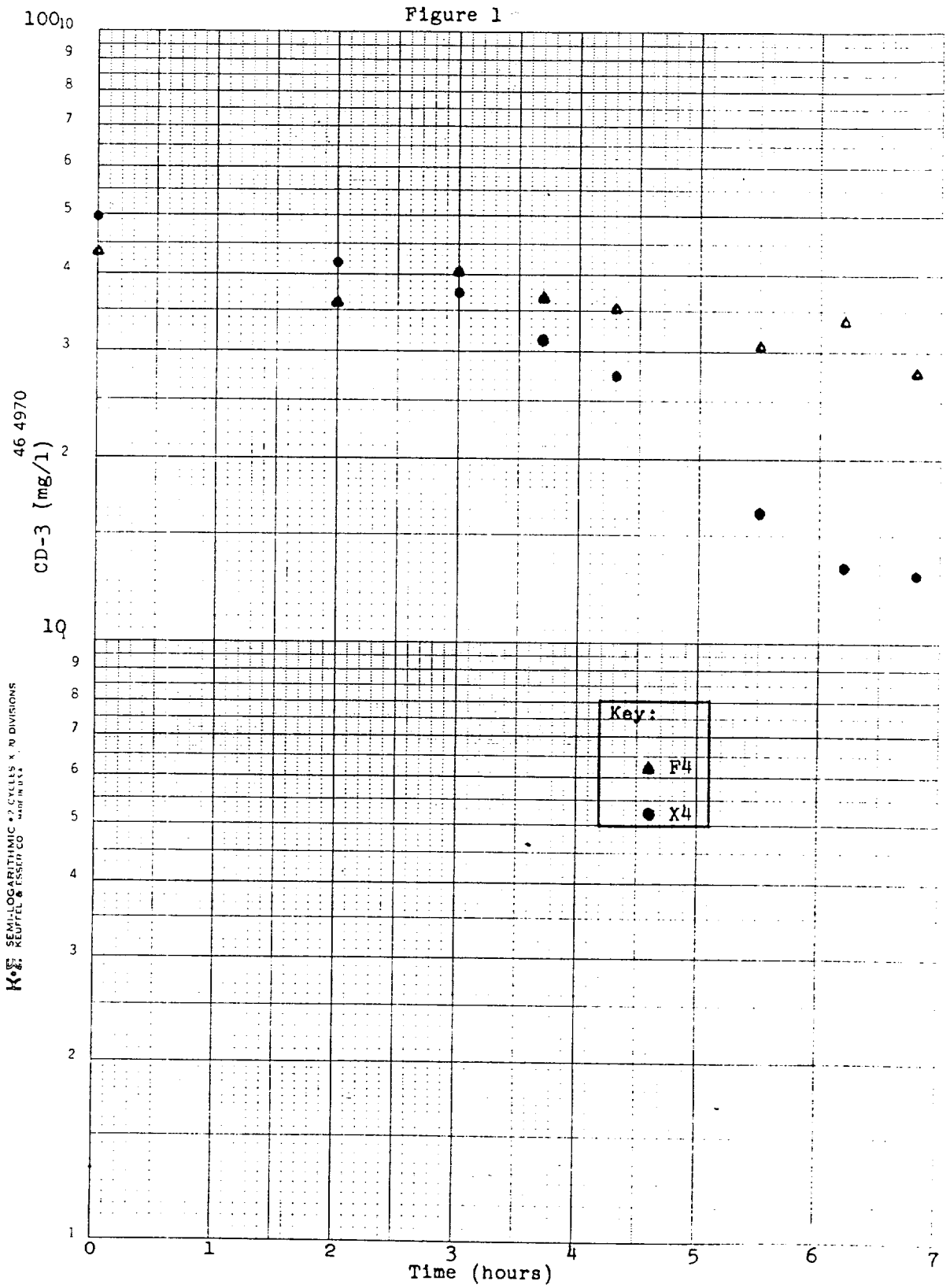

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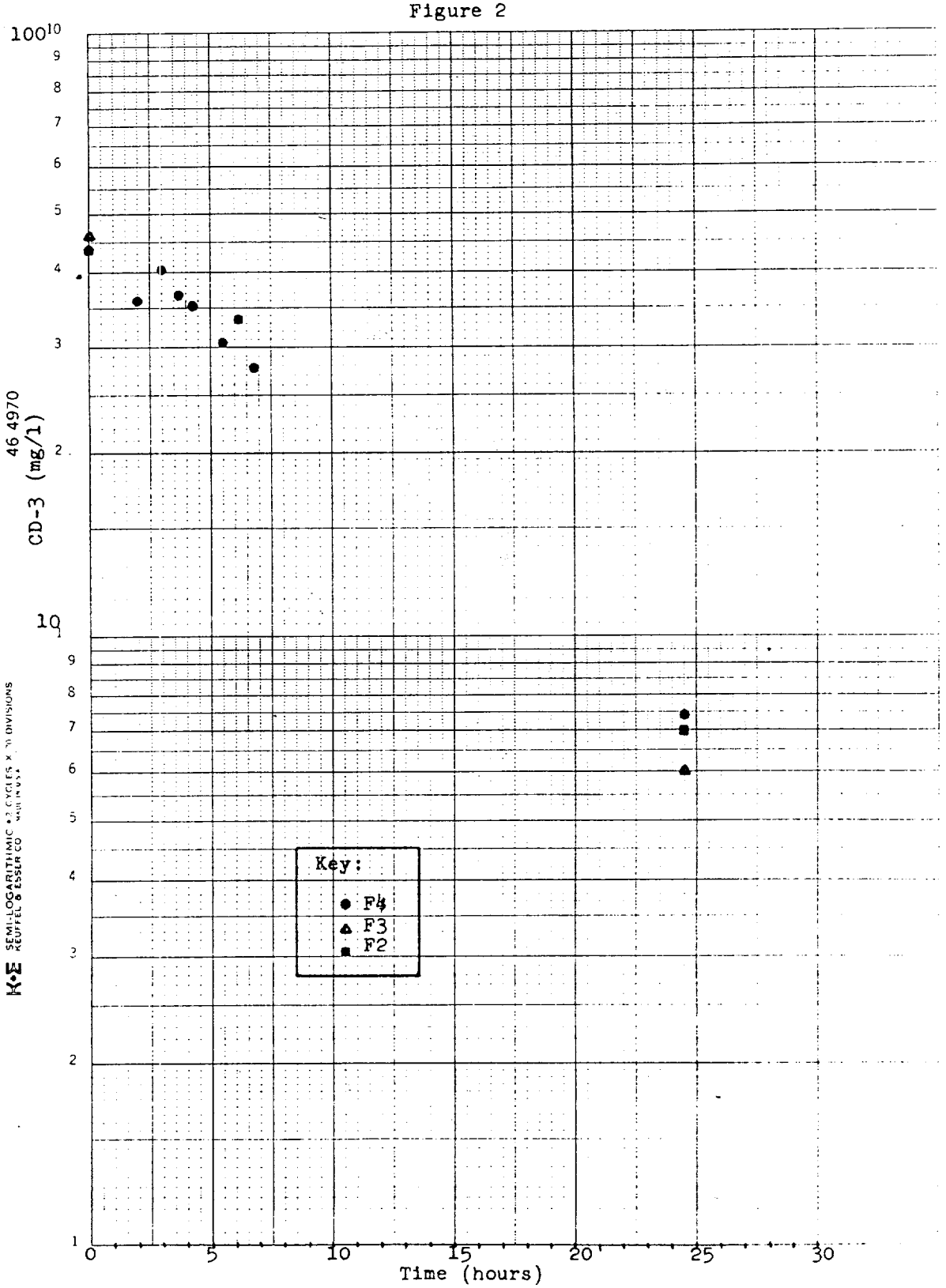
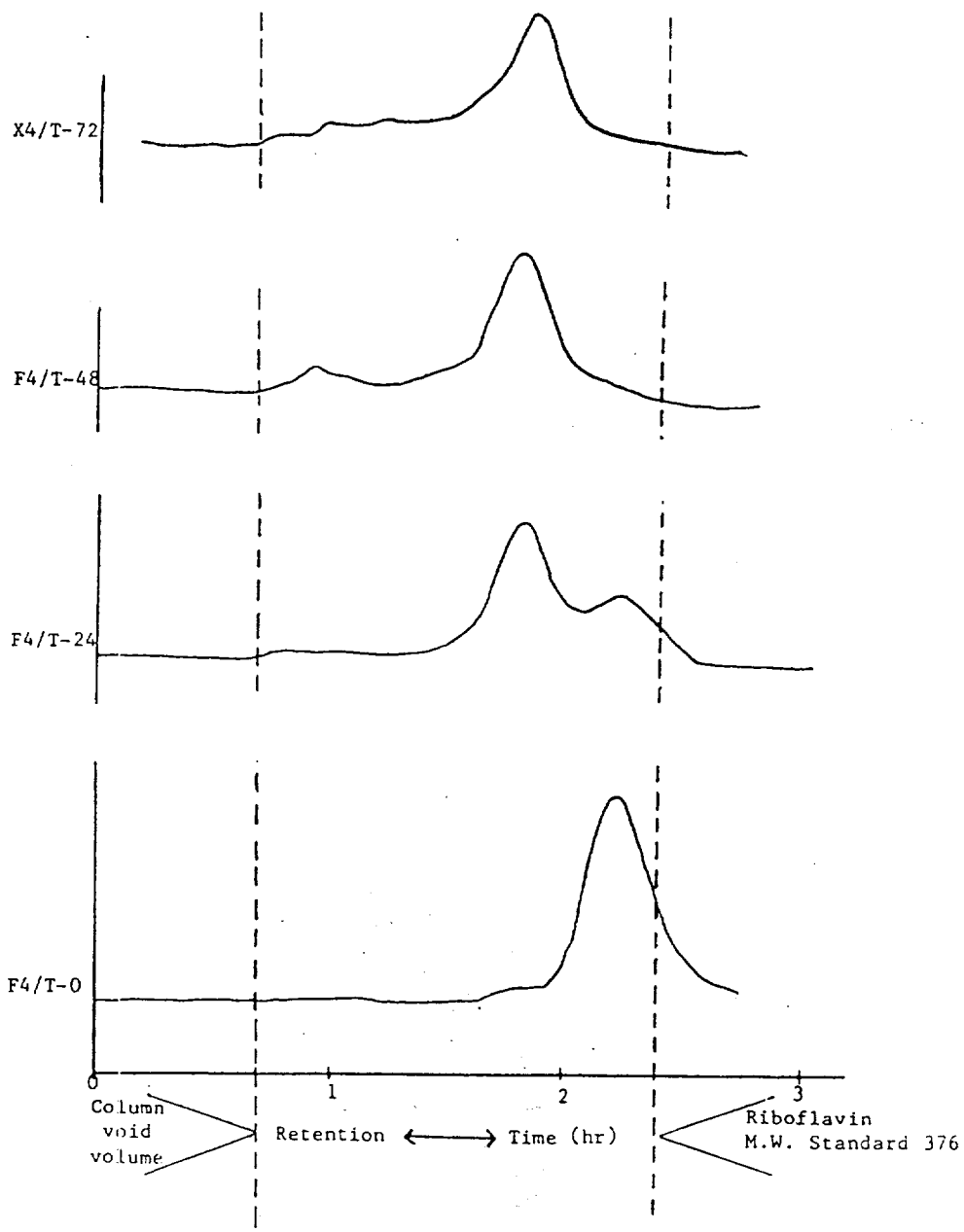
FIGURE 2. Plot of F-2, F-3 and F-4 Concentration of CD-3 (0-25 hr.)

Figure 2



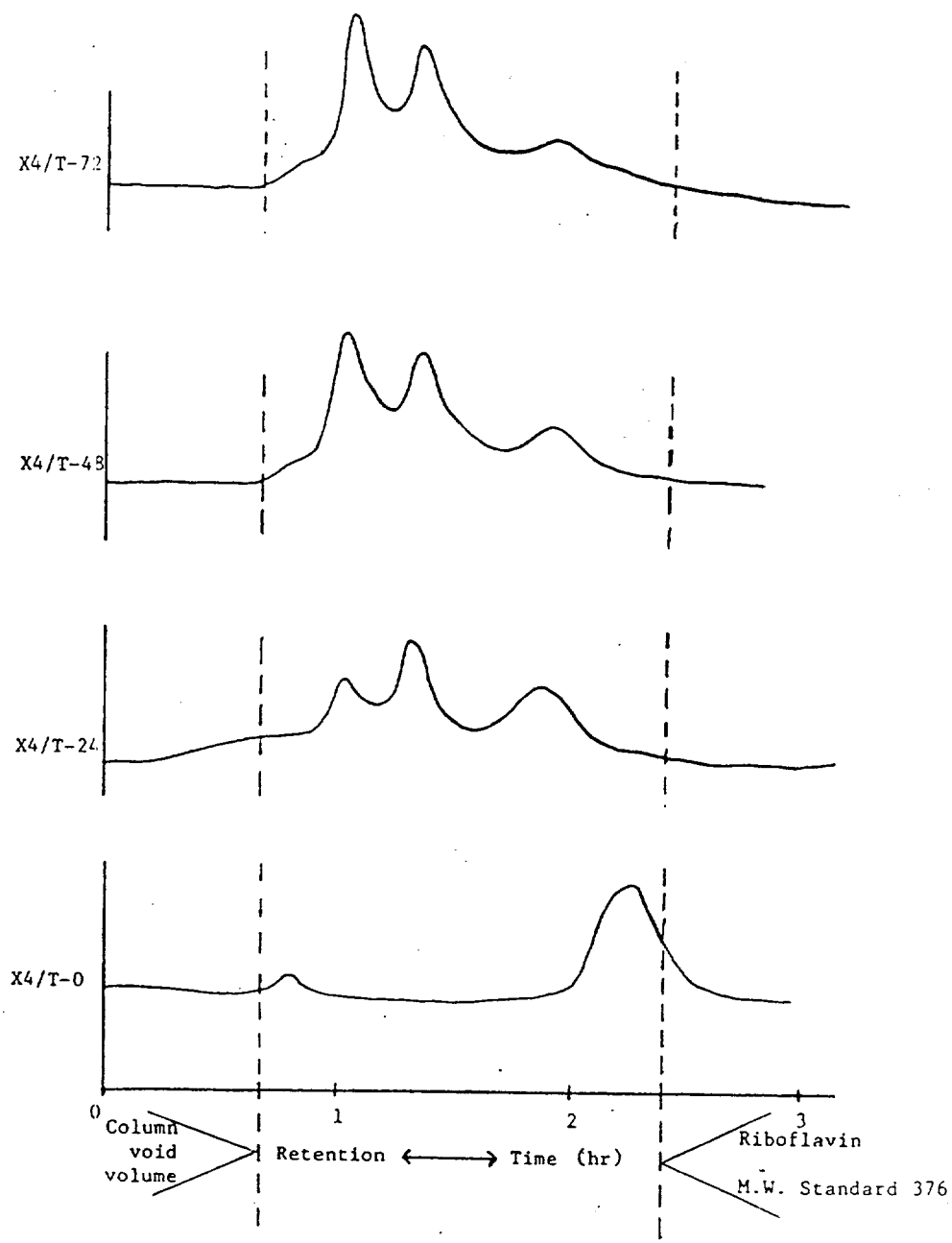
Accession No. 905401 HAEL No. 82-0239

FIGURE 3. Graph of F-4 Test Solution Aqueous Exclusion Chromatography.



Accession No. 905401 HAEL No. 82-0239

FIGURE 4. Graph of X-4 Test Solution Aqueous Exclusion Chromatography.



ATTACHMENT 1. Acute Aquatic Effects Data Forms

Batch 2x 28840-6 START 9:00 AM 4/19/83 Batch 2y 240261 STOP 5:00 AM 4/20/83

Accession No. 905401 | Test Item 82-0239 | CD 3 | Serial No. 00388

ACUTE AQUATIC EFFECTS
HEALTH, SAFETY AND HUMAN FACTORS LAB

Date 4/19/83 | Test Volume 20 L | Concentration 500 mg/L | Starting Conditions ST, X, AR 0.05 L/MIN. Ip: 0.28 G. Ave. Wt. Control C-1 Inner Tank Surface Reading Set At 6000 FC/60 HR Motor Res

Time (HRS)	°C	DO	PH	DM	GF	DT	HT	PP	Remarks
.10	19	7.6	7.9					1.0	P.P. ADDED
.16	23	7.9	7.7					1.0	Test Sol. Temp. Thermo. Reading Changed Slightly
.24	26	7.9	7.8						

Date 4/19/83 | Test Volume 20 L | Concentration 500 mg/L | Starting Conditions ST, X, AR 0.05 L/MIN. Inner Tank Surface Reading Set At 6000 FC/60 HR Motor Response

Time (HRS)	°C	DO	PH	DM	GF	DT	HT	PP	Remarks
.24	26	7.9	7.8					1.0	Test Solution Reading Higher P.P. ADDED
.30	26	7.9	7.8					1.0	

Date 4/19/83 | Test Volume 20 L | Concentration 500 mg/L | Starting Conditions ST, X, AR 0.05 L/MIN. Inner Tank Surface Reading Set At 6000 FC/60 HR Motor Response

Time (HRS)	°C	DO	PH	DM	GF	DT	HT	PP	Remarks
.48	26	7.2	7.8					1.0	Test Solution Reading Higher P.P. ADDED
.54	27	7.5	7.7					1.0	A.D.
.58	27	7.8	7.8					.6	A.D.
.58	27	7.0	7.8					.3	A.D. A.D.
.16	26	7.5	7.7					.2	M
1.10	26	7.4	7.8					.2	M
1.14	27	7.7	7.9					.2	M

Date 4/19/83 | Test Volume 20 L | Concentration 500 mg/L | Starting Conditions ST, X, AR 0.05 L/MIN. Inner Tank Surface Reading Set At 6000 FC/60 HR Motor Response

Time (HRS)	°C	DO	PH	DM	GF	DT	HT	PP	Remarks
.28	27	7.8	7.9					1.0 M	Test Solution Reading Higher P.P. ADDED
.34	26	7.0	7.9					1.0 M	
.36	26	7.8	7.8					1.0 M	
1.10	26	7.9	7.8					1.0 M	
1.14	27	7.7	7.8					1.0 M	
1.18	26	7.7	7.8					1.0 M	

Date | Test Volume | Concentration | Starting Conditions

Time (HRS)	°C	DO	PH	DM	GF	DT	HT	PP	Remarks

Signature of Author [signature] on 4-25 1983 Reviewed By [signature] on 4-26 1983

ATTACHMENT 1. Acute Aquatic Effects Data Forms

ACUTE AQUATIC EFFECTS

HEALTH, SAFETY AND HUMAN FACTORS LAB

Chk Zy Yl Ac Zn 28360.6 START 9:00 AM

Accession No.: | Item No.: -- | Test Item: CONTROL | Material No.: 00389

Date: 2/4/83 | Test Volume: 2.0 L | Concentration: 0 mg/L | Starting Conditions: ST, X, AR 0.05 L/min. AR 0.28 G Ave. Light at start of control C-1

	Time (HRS)	°C	DO	PH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
C-1										
0/0	0	21.4	8.5	7.8					1.0 N	LP ADDED
0/0	1.6	21.1	8.3	7.8					1.0 N	
0/0	2.4	21.3	8.2	7.9					1.0 N	DISSOLVED

Date: 2/4/83 | Test Volume: 2.0 L | Concentration: 0 mg/L | Starting Conditions: ST, X, AR 0.05 L/min.

	Time (HRS)	°C	DO	PH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
C-2										
0/0	2.4	21.3	8.3	8.0					1.0 N	LP ADDED
0/0	3.0	21.3	8.2	7.9					1.0 N	
0/0	4.8	21.3	8.1	7.9					1.0 N	DISSOLVED

Date: 2/4/83 | Test Volume: 2.0 L | Concentration: 0 mg/L | Starting Conditions: ST, X, AR 0.05 L/min.

	Time (HRS)	°C	DO	PH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
C-3										
0/0	4.8	21.3	8.1	8.0					1.0 N	LP ADDED
0/0	5.4	21.4	7.9	8.0					1.0 N	
0/0	7.2	21.4	8.2	8.0					1.0 N	
0/0	7.8	21.4	8.2	8.0					1.0 N	
0/0	9.6	21.4	8.4	8.0					1.0 N	
0/0	10.2	21.4	8.3	8.0					1.0 N	
0/0	11.4	21.4	8.1	8.0					1.0 N	

Date: 2/4/83 | Test Volume: 2.0 L | Concentration: 0 mg/L | Starting Conditions: ST, X, AR 0.05 L/min.

	Time (HRS)	°C	DO	PH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
C-4										
0/0	7.2	21.4	8.1	8.1					1.0 N	LP ADDED
0/0	7.8	21.4	8.3	8.0					1.0 N	
0/0	9.6	21.4	8.0	7.9					1.0 N	
0/0	10.2	21.4	8.0	7.9					1.0 N	
0/0	11.4	21.4	8.0	7.9					1.0 N	
0/0	16.8	21.4	8.0	7.9					1.0 N	

Date: | Test Volume: | Concentration: | Starting Conditions:

	Time (HRS)	°C	DO	PH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks

Signature of Author: [illegible] on 4/25/83 19 83 Reviewed By [illegible] on 4/26 19 83

NFO-00325-210 (12/80)

START 9:00 AM.

Accession No. 905401 | Test Item: C.D-3 | Serial No. 00390

ACUTE AQUATIC EFFECTS

HEALTH, SAFETY AND HUMAN FACTORS LAB

F-1 — Date: 4/18/83 | Test Volume: 2.0 L | Concentration: 5.0 mg/L | Starting Conditions: ST, E, AR 0.05 L/min. pH: 0.25 G Air W.T. Control Cl. Air/Water Reading Set At: 60 FC/20 mA

Time (hrs)	°C	DO	pH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
0	19	8.6	7.8					1.0	L.P.: A.D.D.E.S.D.
16	8.0	7.9	7.3					0	Test Solution Turned Reddish-Purple Color
24	21.0	8.2	8.0						

F-2 — Date: 4/18/83 | Test Volume: 2.0 L | Concentration: 5.0 mg/L | Starting Conditions: ST, E, AR 0.05 L/min. Air/Water Reading Set At: 60.0 FC/20 mA

Time (hrs)	°C	DO	pH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
24	21.0	8.4	8.0					1.0	Test Solution Reddish-Purple. L.P.: A.D.D.E.S.D.
30	21.0	8.2	7.9					0	

F-3 — Date: 4/18/83 | Test Volume: 2.0 L | Concentration: 5.0 mg/L | Starting Conditions: ST/E/AR 0.05 L/min. Air/Water Reading Set At: 60.0 FC/20 mA

Time (hrs)	°C	DO	pH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
48	19	8.5	7.9					1.0	Test Solution Reddish-Purple L.P.: A.D.D.E.S.D.
54	19	8.3	7.9					0	
72	19	8.5	7.9						

F-4 — Date: 4/18/83 | Test Volume: 2.0 L | Concentration: 5.0 mg/L | Starting Conditions: ST/E/AR 0.05 L/min. Air/Water Reading Set At: 60 FC/20 mA

Time (hrs)	°C	DO	pH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks
72	19	8.5	7.9					1.0	Test Solution Reddish-Purple L.P.: A.D.D.E.S.D.
78	19	8.5	7.9					0	

F-5 — Date: / | Test Volume: | Concentration: | Starting Conditions:

Time (hrs)	°C	DO	pH	DM Observations	GF Observations	DT Observations	HT Observations	PP Observations	Remarks

Signature of Author: [illegible] on 4-25 1983 Reviewed By: [illegible] on [illegible] 19[illegible]

Accession No. 905401

ATTACHMENT 1. Acute Aquatic Effects Data Forms

HAEL No. 82-0239

Accession No. 905401 HAEL No. 82-0239

ATTACHMENT 2. Summary of Chemical Characterization of Dilution Water

Chemical Characteristics of Dilution Water Date of Sample: 02/09/83

(Values listed below are as mg/L unless otherwise noted.)

Total Metals by ICP/AES:		Water Quality Parameters:	
Aluminum	<(0.3)	pH (determined @ 20°C)	7.8
Arsenic	<(0.001)	Conductivity, as µmhos/cm	357
Barium	0.02	Alkalinity (as $CaCO_3$)	94
Cadmium	<(0.005)	Total Hardness (as $CaCO_3$)	130
Chromium	<(0.02)	Non-carbonate Hardness (as $CaCO_3$)	36
Cobalt	<(0.02)		
Copper	<(0.01)	Total Residual Cl_2, as µg/L	< 100
Iron	<(0.01)	Total Dissolved Solids	159
Lead	<(0.3)	Molybdenum-Reactive Dissolved SiO_2	0.5
Manganese	0.01		
Nickel	<(0.04)	Total NH_3 (as N)	ND(0.05)
Silver	<(0.01)	Organic Nitrogen	0.19
Zinc	<(0.01)	Nitrates (as N)	0.4
		Nitrites (as N)	ND(0.01)
		Soluble Halides (as Cl^-)	24
		Fluoride	0.1
		Soluble Sulfate	28
		Total Cyanide	0.001
		Total Phosphorus	< 0.02

Key:

ND( ) = Not Detected at (Method Detection Limit)

<( ) = Less than the (Limit of Quantification)

Accession No. 905401

HAEL No. 82-0239

ATTACHMENT 3. Quality Assurance Unit Statement.

Q.A. INSPECTION STATEMENT
(CFR 58.35(B)(7))

PAGE 1
07/24/87

STUDY: 82-0239-1 STUDY DIRECTOR: ROBILLARD,K.A.
ACCESSION NUMBER: 905401

STUDY TYPE: ACUTE AQUATIC EFFECTS TEST(FATHEAD MINNOW-FL.)

M. Susan James
(AUDITOR, QUALITY ASSURANCE UNIT)

7/24/87
DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF THE HAEL, EASTMAN KODAK COMPANY, ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
07/23/87	FINAL REPORT REVIEW	07/23/87

Accession No. 905401

HAEL No. 82-0239

DIS No.: 230906R

Prepared by William S. Ewell

Date 7/22/87

William S. Ewell
Principal Investigator-Biology
Eco-Chem Testing Group

Prepared by Rodney J. Boatman

Date 7/22/87

Rodney J. Boatman
Principal Investigator-Chemistry
Toxicological Sciences Section

Approved by Joseph W. Gorsuch

Date 7/22/87

Joseph W. Gorsuch
Group Leader
Eco-Chem Testing Group

Approved by T J Dagon

Date 7/23/87

Thomas J. Dagon
Unit Director
Environmental Sciences Section

Reviewed by M. L. James

Date 7/23/87

Quality Assurance Unit
Health and Environment Laboratories

Approved by Kenneth A. Robillard

Date 7/23/87

Kenneth A. Robillard
Study Director
Eco-Chem Testing Group

0174E

Environmental Effects and Fate of Color Developers

Introduction

Color developers are para-phenylenediamine molecules that react with silver and couplers to produce dyes in photographic media. As such, the color developers are an integral part of color film and paper processing. These chemicals are designed to be reactive to accomplish the task of image making, and this reactivity is also responsible for effects on some environmental systems, as well as a lack of persistence in the environment. This paper will describe the environmental effects and fate of some of the color developers. It is assumed that all color developers display the reactivity measured for the most common examples, because of the similarities in structure among the group. This paper will focus on color developer-3 (CD-3; N-[2-[4-Amino-3-methylphenyl)ethylamino]ethyl]-methanesulfonamide sesquisulfate monohydrate ; CAS # 25646-71-3) and color developer-4 (CD-4; 2-[(4-Amino-3-methylphenyl)ethylamino]ethanol sulfate; CAS # 25646-77-9). It should be noted that color developers are chemically altered as part of the development process, producing sulfonated materials that are of lower toxicity than the parent color developers. The primary issue of this paper in the effects and fate of the CD's themselves, especially in relation to EU environmental classification, therefore the sulfonates will not be covered in detail.

Aquatic Effects

There have been many studies on the aquatic effects of the color developers. Because of the variability of test waters and of organisms within a species, these studies have not resulted in a single LC_{50} value for any organism. The values for both *Daphnia magna* (daphnia) and *Pimephales promelas* (minnow) were in the range of 0.1-1 mg/L (CD-4) and 1-10 mg/L (CD-3) in testing conducted at Eastman Kodak Company. Other published results may vary slightly from these values, as discussed above. These values correspond to the risk phrase R50 (Very toxic to aquatic organisms) for CD-4, and R51 (Toxic to aquatic life) for CD-3. Both published data and Kodak studies suggest that the color developers may be inhibitory to unacclimated wastewater treatment microorganisms at concentrations around 100 mg/L, however, this effect is diminished when the organisms are acclimated to the chemical.

Environmental Fate

The log of the octanol/water partition coefficient for CD-3 has been estimated to be 0.67 by an high performance liquid chromatography technique. Log $P_{OW} < 3$ are not considered to be concerns for bioconcentration. Biochemical oxygen demand and biodegradation studies suggest that microbial degradation is not an important environmental removal mechanism for color developers.

Contributions from thermal, oxidative and photochemical degradation processes are far more important in the determination of the ultimate fate of these chemicals. In an analysis of thermal effects, experiments determined that 90% of CD-4 was degraded in 6, 12, and 20 hours at 30, 20, and 15° C, respectively. The degradation seemed to be accelerated by the formation of initial degradation products (Giordano and Robillard, unpublished data, Eastman Kodak Company, 1989). Similar results are seen in oxidative treatment. Solutions originally containing 1000 mg/L of CD-3 or CD-4 contained no detectable color developer after two hours of aeration, followed by overnight standing. The decomposition products were difficult to characterize, and were thought to be polymeric (Richardson, Thames Water Directorate of Scientific Services, Catchment Quality Control Report No. 8, 1981). These results are consistent with work done at Kodak on CD-3 (unpublished results summarized in Summary of Environmental Testing, 1983), in which solutions containing 50 and 500 mg/L of CD-3 were aerated at 0.2 ft^3/hour for 48 hours. The degradation of the CD-3 corresponded to a half-life of 10.5 hours. There was no loss of CD-3 from a control vessel sparged with nitrogen.

The photochemical degradation of CD-4 has been studied using xenon-arc lamp lighting (Giordano and Robillard, unpublished results, Eastman Kodak Company, 1989). At approximately 23° C and pH 7, samples of CD-4 exposed to 6145 foot-candles of visible light (400-700 nm) had a half life of 0.6 hours. Dark control samples had a calculated half life of 5.6 hours, indicating that other processes (oxidation, for example) were in competition with photodegradation. The toxicity of the photolysis decomposition products has been studied for both CD-3 and CD-4. Solutions of 50 mg/L of CD-3 or CD-4 illuminated under xenon-arc lighting for 72 hours, displayed no toxicity to the fathead minnow in an additional 72-hour period (Ewell and Boatman, unpublished data, Eastman Kodak Company, 1987; Ewell and O'Boyle, unpublished data, Eastman Kodak Company, 1988)

The risk phrase R53 (May cause long-term adverse effects in the aquatic environment) is assigned to substances that do not degrade or have log P_{OW} >3. The criteria for degradation include A) passing a test for ready biodegradability, B) having a BOD/COD ratio in excess of 0.5 or C) having other convincing scientific evidence to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level of > 70% within a 28-day period. The evidence described above shows that the color developers are degradable according to criterion C and thus should not take the risk phrase R53.

Summary

Color developers are acutely toxic to some aquatic species, however they are not persistent in the environment nor do they bioaccumulate. Long-term adverse effects are not likely. The data supports R50 or R51, but not R53.

C.J. Ruffing
October 6, 1994

ENVIRONMENTAL SAFETY DATA SHEET

HEALTH SAFETY AND HUMAN FACTORS LABORATORY

ENVIRONMENTAL SCIENCES GROUP

QUESTIONS CONCERNING DATA ON THIS SHEET SHOULD BE REFERRED TO THE ENVIRONMENTAL SCIENCES GROUP, EXTENSION: 85211

CAS REG. NO.	HSHFL NO.	MOLECULAR WT.	DATE	ACCESSION NO.
24567-76-8[1]	74-0243 & 82-0239	436.5	5/11/82 Rev. 2/3/83	905401

EMPIRICAL FORMULA

$C_{12}H_{21}N_3O_2S \cdot 3/2H_2SO_4 \cdot H_2O$

SRID #

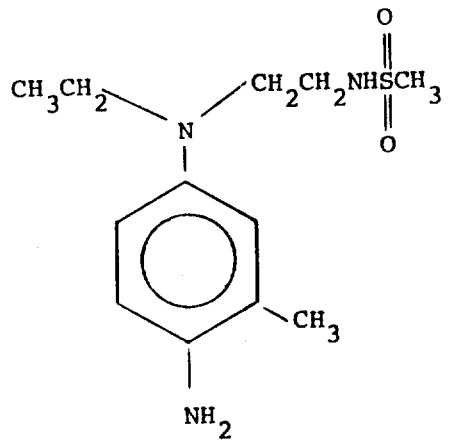
COMPOUND NAME

N-(2-((4-Amino-3-methylphenyl)ethylamino)ethyl)methanesulfonamide Sulfate (2:3) Monohydrate[2]

SYNONYMS

CD-3; Color Developing Agent CD-3; D109B; D_2W

STRUCTURE



$\cdot 3/2H_2SO_4 \cdot H_2O$

TSCA INVENTORY LIST ☒ YES ☐ NO

REGULATORY STATUS

PHYSICAL CHEMICAL PROPERTIES

MELTING POINT 123-124°C (by DSC)

BOILING POINT

VAPOR PRESSURE mm@ °C

PH of 0.1% AQUEOUS SOLUTION

PKa 2.64, 6.49 **Ka**

OTHER Threshold of thermal degradation is 162°C

SOLUBILITY

H_2O: 300 g/L (distilled water)

OCTANOL

CORN OIL

ACETONE

DMSO

OTHER: in water @ pH 10.0: 18 g/L[6]

REMARKS

Absorption spectrum pH: 2.0 6.5 11.7

λmax (nm), ε(liter/mole-cm) 239(1.89 x 10^3) 239(1.06 x 10^4) 253(1.34 x 10^4)

OXYGEN DEMAND TESTS

TOD	COD	BOD_5	BOD_{20}
1.58g/g[3,4]; 1.30g/g[5]	0.90g/g[3,5]; 1.14g/g[4]	.095g/g[3]; 0.1g/g[5]	

REMARKS The BOD_5 reported for an acclimated sludge is 0.391 g/g.[4] Chemical oxidation with pure oxygen required 0.176 g of oxygen/g of CD-3. The BOD_5 measurements, therefore, represent simple oxygen demand rather than any biological degradation.[4]

ENVIRONMENTAL HALF-LIFE

T ½ BIOLOGICAL See Reference/Comments Section.

PRODUCTS OF BIODEGRADATION OR BIOTRANSFORMATION

T ½ PHOTOCHEMICAL (fc @ nm)

PRODUCTS OF PHOTODEGRADATION

T ½ HYDROLYSIS

PRODUCTS OF HYDROLYSIS

REMARKS T 1/2 Oxidation in water: 10.5 -19.1 hrs. Several possible oxidative pathways are believed to be involved resulting in oligomers and humic-type compounds.

FILE NO. 74-0243 & 82-0239

ENVIRONMENTAL SAFETY DATA SHEET
HEALTH SAFETY AND HUMAN FACTORS LABORATORY
ENVIRONMENTAL SCIENCES GROUP

ACCESSION NO. 905401

ACUTE AQUATIC EFFECTS

LC_{50} - PIMEPHALES PROMELAS (FATHEAD MINNOW)	1-10 mg/L*	LC_{50} - HELISOMA TRIVOLVIS (SNAIL)	10-100 mg/L*
LC_{50} - DAPHNIA MAGNA (WATER FLEA)	1-10 mg/L*	LC_{50} - ASELLUS INTERMEDIUS (PILL BUG)	10-100 mg/L*
LC_{50} - GAMMARUS FASCIATUS (SIDESWIMMER)	10-100 mg/L*	LC_{50} - LUMBRICULUS VARIEGATUS (SEGMENTED WORM)	10-100 mg/L*
LC_{50} - DUGESIA TIGRINA (FLATWORM)	1-10 mg/L*		

REMARKS

*Results of static 96 hour exposures to nominal test article concentrations of 100, 10 and 1 mg/L.

SECONDARY WASTE TREATMENT COMPATIBILITY

IC_{50} 150 mg/L

REMARKS

The test article was shown to be inhibitory to conventional waste treatment systems at a level of 100 mg/L.[4]

BIO-CONCENTRATION POTENTIAL

WLN

OCTANOL/H_2O COEFF. log P = 0.67*

T ½ - CLEARANCE

BIOCONCENTRATION FACTOR

REMARKS

*This value was determined at pH 7.5 using an HPLC procedure.

PLANT GROWTH EFFECTS NO EFFECT CONCENTRATION

GERMINATION		SEEDLING	
LOLIUM PERENNE (RYEGRASS)	100 mg/L	ZEA MAYS (CORN)	100 mg/L
RAPHANUS SATIVUS (RADISH)	10 mg/L	TAGETES PATULA (MARIGOLD)	100 mg/L
LACTUCA SATIVA (LETTUCE)	10 mg/L	LACTUCA SATIVA (LETTUCE)	100 mg/L
OTHER		RAPHANUS SATIVUS (RADISH)	100 mg/L

REMARKS

The green alga, Selenastrun capricornutum was inhibited by 4.0 mg/L of the test article, but not by 0.4 mg/L.[4]

REFERENCES/COMMENTS

Acute Aquatic Effects (cont.):

A dynamic 96 hour toxicity test was performed using Pimephales promelas as the test organism. Mean measured test exposures were: 8.4, 5.7 and 2.0 mg/L. The LC_{50} value determined in this test is in the interval 5-10 mg/L.

T 1/2 Biological:

Aerobic - In a 21 day test using acclimated sludge microorganisms no significant degradation producing CO_2 was observed.

Anaerobic - No biological degradation was observed in a 56 day test as measured by the cumulative evolution of methane and CO_2.

ENVIRONMENTAL SAFETY EVALUATION

Tier Testing Ratings (Environmental Risk Analysis for Chemicals, "Sequential Testing for Chemical Risk Assessment", in publication)	LOW (1)	MED (2)	HIGH (3)
PERSISTENCE	1		
SECONDARY WASTE TREATMENT EFFECTS			3
ACUTE AQUATIC EFFECTS			3
BIOCONCENTRATION	1		

PREPARED BY

Philip W. Jenkins/Joseph W. Gorsuch/Rodney J. Boatman

DATE 5/11/82 Revised 2/3/82

WES-910 (8/81)

Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-3617
United States of America
Telephone No. (716) 588-5447

ENVIRONMENTAL SAFETY DATA SHEET

CAS No. 25646-71-3
SRID No.

Accession No. 905401
HAEL No. 74-0243
HAEL No. 82-0239

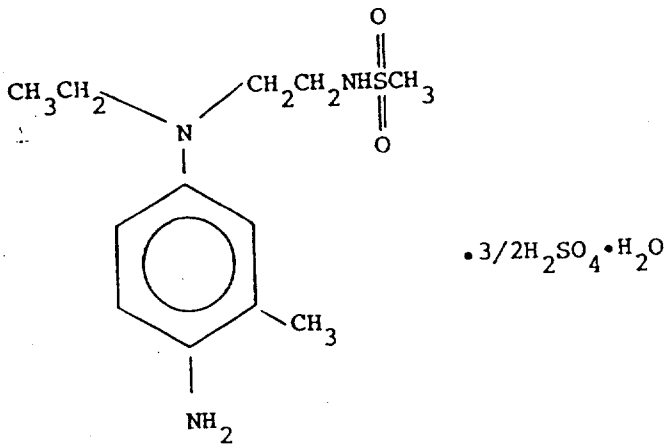
PM 1572

COMPOUND NAME(s) (Name from request sheet is first):
N-(2-((4-Amino-3-methylphenyl)ethylamino)ethyl)methanesulfonamide Sulfate (2:3) Monohydrate;
CD-3; Color developing Agent CD-3; D109B; D_2W

Molecular Formula: $C_{12}H_{21}N_3O_2S \cdot 3/2H_2SO_4 \cdot H_2O$

Molecular Wt.: 436.5

Structure:



ENVIRONMENTAL EVALUATION [1]

		Low(1)	Med(2)	High(3)
PERSISTENCE		1		
ABIOTIC FATE	Oxidative Degradation			3
BIOLOGICAL FATE	Bioconcentration	1		
	Biodegradation	1		
BIOLOGICAL EFFECTS	Acute Aquatic			3
	Secondary Waste Treatment Compatibility			3

PHYSICAL-CHEMICAL PROPERTIES

Solubility:
Water: 380g/L **Others:** In water at pH 10.0: 18g/L

Oxygen Demand:
COD: 0.90g/g[3,5]
1.14g/g[4]
BOD_5: 0.095g/g[3]
0.1g/g[5]

Other:
B.P.: Thermal degradation at 162°C **M.P.:** 123 - 124°C
Dissociation Constant: pK_1 = 2.64; pK_2 = 6.48

BIODEGRADATION

A 21-day test for Aerobic biodegradability using acclimated sludge microorganisms as the inoculum showed no significant degradation of the test article as measured by the evolution of CO_2.

Remarks: A 56-day test for Anaerobic biodegradability using acclimated sludge microorganisms as the inoculum showed no significant degradation of the test article as measured by the cumulative evolution of methane and CO_2.

BIOCONCENTRATION

Remarks: Log P (Octanol/Water) = 0.67 determined at pH 7.5 using an HPLC procedure.

CHEMICAL DEGRADATION

Remarks: $T_{1/2}$ in water under oxidative conditions 10.5-19.1 hours.

AQUATIC EFFECTS

Test Species	Test Duration	EC_{50}*/LC_{50}** Point Estimate	No Observed Effect Concentration (Acute)
Daphnid *Daphnia magna* (Dm)	96 hr-A	3.2 mg/L	1.0 mg/L
Fathead Minnow *Pimephales promelas* (Pp)	96 hr-A	1.8 mg/L	0.1 mg/L
Aquatic Earthworm *Lumbriculus variegatus* (Lv)	96 hr-A	3.2 mg/L	1.0 mg/L
Sideswimmer *Gammarus fasciatus* (Gf)	96 hr-A	18 mg/L	0.1 mg/L

AQUATIC EFFECTS (continued)

Test Species	Test Duration	EC_{50}*/LC_{50}** Point Estimate	No Observed Effect Concentration (Acute)
Pillbug *Asellus intermedius* (Ai)	96 hr	32 mg/L	10 mg/L
Snail *Helisoma trivolvis* (Ht)	96 hr	32 mg/L	1 mg/L
Flatworm *Dugesia tigrina* (Dt)	96 hr	3.2 mg/L	1 mg/L

*EC_{50} = Effect Concentration based on immobilization of 50% of population for the daphnids only.
**LC_{50} = Lethal Concentration to 50% of the population.

Remarks: A dynamic 96 hour toxicity test was performed using *Pimephales promelas* as the test organism. Mean measured test exposures were: 8.4, 5.7, and 2.0 mg/L. The LC_{50} value determined in this test is the interval 5-10 mg/L.

SECONDARY WASTE TREATMENT COMPATIBILITY

5-hr IC_{50}* = 150 mg/L

*IC_{50} = The concentration of chemical that inhibits the metabolism of [^{14}C]glucose by secondary waste treatment microorganisms by 50% as measured by [^{14}C]carbon dioxide evolution.

Remarks: The test article was shown to be inhibitory to conventional waste treatment system at a level of 100 mg/L

PLANT GROWTH EFFECTS

GERMINATION	Test Duration	No Observed Effect Concentration (Adverse)
Ryegrass (*Lolium perenne*)	7 Days	100 mg/L
Radish (*Raphanus sativus*)	7 Days	10 mg/L
Lettuce (*Lactuca sativa*)	7 Days	10 mg/L
SEEDLING		
Corn (*Zea mays*)	7 Days	100 mg/L
Marigold (*Tagetes patula*)	7 Days	100 mg/L
Lettuce (*Lactuca sativa*)	7 Days	100 mg/L
Radish (*Raphanus sativus*)	7 Days	100 mg/L

Remarks: The green alga *Selenastrun capricornutum* was inhibited by 4.0 mg/L of the test article, but not by 0.4 mg/L.

REFERENCES

1. Astill, B.D., et al., "Sequential Testing for Chemical Risk Assessment", in "Environmental Risk Analysis For Chemicals", Conway, R.A., Ed., Van Nostrand Company, New York, NY, 1982, pp. 412-433.

Prepared by: Charles J. Ruffing Date: August 19, 1992

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APR 12 1982

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134781R
TX-82-07

Evaluation of the Carcinogenic Potential in Hamsters Following Intratracheal Administration of CD-3

Milan S. Vlaovic, D.V.M., Ph.D.
Toxicology Section

May 8, 1981

The carcinogenic potential of 4-amino-3-methyl-N-ethyl-N-(β-methylsulfonamidoethyl)anilinesesguisulfate monohydrate (CD-3) (Acc. No. 905401) was evaluated in hamsters. The study consisted of intratracheal administration of the test chemical. The testing facility was the Eppley Institute for Research in Cancer, University of Nebraska Medical Center at Omaha, Nebraska, under the sponsorship of Eastman Kodak Company. The intratracheal study was conducted in compliance with the protocol accepted by Eastman Kodak Company on June 21, 1973. (Addendum).

TEST MATERIALS

A representative sample of the test compound was submitted to the Eppley Institute by Eastman Kodak Company. It was accompanied by analytical data, acute toxicity data and a health hazard summary that was originated in the Health, Safety, and Human Factors Laboratory.

Intratracheal Study in Hamsters

ANIMALS: Male and female random bred Syrian golden hamsters from Lakeview Hamsters, New Field, New Jersey, were used for this study. At the start of the treatment the hamsters were about 8 weeks of age.

HOUSING: The two compound treatment groups (0.25 mg or 0.50 mg) each contained 30 males and 30 females. They were selected at random and housed six per cage. The vehicle control group (VC) contained 90 males and 90 females housed six per cage which were treated intratracheally with 0.2 ml of normal saline once per week for

30 weeks. In addition there were two untreated control groups (UUC, UUB). Each untreated control group contained 48 males and 48 females housed six per cage.

The animals were maintained in a temperature and humidity controlled room on corn cob bedding (Bed-O'-Cobs) supplied by SASCO, Inc., 612 N. 16th Street, Omaha, Nebraska. Wayne Lab Blox pellets (Allied Mills, Inc., Chicago, Illinois) and tap water from the Omaha City supply were available ad libitum throughout the experiment.

TREATMENT: Hamsters were treated according to the intratracheal procedure described by Saffiotti, et al. (Cancer Research 28:104-124, 1968). Intratracheal instillation of the test and control chemicals was performed by cannulation under the epiglottis into the tracheal lumen under sodium Brevitol™ anesthesia. Hamsters receiving the low dose (0.25 mg) of CD-3 were treated once a week for thirty weeks. High doses (0.50 mg) of the CD-3 were administered once a week for fifteen weeks. Following treatment, the animals were maintained on basal diet and water ad libitum for 20 months. The vehicle (saline) control group was treated with saline by the same method.

SURVIVAL: Tables 1-10 and graphs 1 and 2 present survival rates for untreated control, vehicle control and treated animals. The data illustrate 50% or greater survival rates after one year for all except the UUB female control group (49%).

OBSERVATIONS: Animals were observed daily for mortality and changes in appearance and behavior. Body weights were recorded weekly

and are presented for the untreated control and intratracheally treated groups in Tables 1-10 and Graphs 3 and 4. There was a trend in hamsters to plateau in weight near the twelfth experimental week; however, the males maintained their peak body weight four to five months longer than female hamsters before weight losses ensued.

PATHOLOGY: All hamsters found dead or killed were necropsied. The trachea was ligated and the lungs removed *en bloc*, expanded by air and fixed by immersion in 10% buffered formalin. All tumors and other gross lesions, a section of each lobe of the lung, larynx, trachea, stem bronchi, liver, kidney, adrenal, and bone marrow were examined routinely. No compound related changes were observed on gross pathology (Tables 11 and 12). Tables 13 and 14 present the neoplasm incidence for hamsters in the two untreated control groups, the groups treated intratracheally with the saline vehicle control and the groups treated with CD-3. No compound related neoplasms were observed.

Tables 15-17 present the summary of histopathologic incidence for hamsters from the two untreated control groups, the VC group and the groups receiving CD-3. Statistically ($p \leq 0.05$) and biologically significant change in the lungs of both treated group males (15/27 and 10/26) was characterized as alveolar cell metaplasia. This change was consistent with the type of treatment, dose, treatment schedule and the irritating nature of the compound. However, this type of lesion is commonly observed in untreated hamsters and is usually provoked by irritation. In addition, tracheal hyperplasia

occurred in male hamsters in low dose CD-3 treated group. Epithelial hyperplasia of the larynx was also observed in both treated groups of female hamsters. The incidence of both changes was statistically significant only when compared to untreated control groups ($p \leq 0.05$). The same changes were produced by saline (vehicle control group) and their incidence was not statistically different ($p \leq 0.05$) from either of the treatment dose groups; they were considered biologically insignificant.

MSV:mlt

Table 1

Mean Average Weights and Percent Survival in Untreated Control Group I

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
0	88.2	48.	100.0	98.7	48.	100.0
1	93.2	48.	100.0	105.0	48.	100.0
2	102.3	48.	100.0	111.6	48.	100.0
3	109.6	48.	100.0	119.0	48.	100.0
4	116.0	48.	100.0	127.0	48.	100.0
5	118.4	48.	100.0	130.0	48.	100.0
6	122.3	48.	100.0	134.2	48.	100.0
7	125.5	48.	100.0	138.5	48.	100.0
8	129.6	48.	100.0	142.5	48.	100.0
9	132.5	48.	100.0	145.1	48.	100.0
10	131.9	48.	100.0	145.3	48.	100.0
11	133.6	48.	100.0	145.7	48.	100.0
12	0.0	48.	100.0	0.0	0.	0.0
13	136.4	48.	100.0	147.8	48.	100.0
14	136.6	48.	100.0	148.2	48.	100.0
15	137.3	48.	100.0	147.9	48.	100.0
16	139.5	48.	100.0	151.7	48.	100.0
17	140.1	48.	100.0	152.5	48.	100.0
18	141.1	48.	100.0	151.6	48.	100.0
19	140.0	48.	100.0	151.1	48.	100.0
20	141.4	48.	100.0	153.5	48.	100.0
21	141.4	48.	100.0	152.6	48.	100.0
22	141.3	48.	100.0	155.5	48.	100.0
23	141.3	48.	100.0	155.6	48.	100.0
24	141.8	48.	100.0	157.1	48.	100.0
25	142.0	48.	100.0	156.8	48.	100.0
26	140.6	48.	100.0	156.9	48.	100.0
27	139.9	48.	100.0	156.5	46.	95.8
28	141.6	47.	97.9	155.6	45.	93.8
29	142.0	47.	97.9	156.4	45.	93.8
30	141.6	47.	97.9	157.0	45.	93.8
31	140.6	45.	93.8	158.0	44.	91.7
32	140.0	45.	93.8	159.5	44.	91.7
33	139.8	45.	93.8	160.8	44.	91.7
34	138.5	45.	93.8	160.3	44.	91.7
35	138.2	45.	93.8	159.7	44.	91.7
36	138.2	45.	93.8	159.3	44.	91.7
37	138.8	45.	93.8	159.9	44.	91.7
38	138.1	45.	93.8	160.0	43.	89.6
39	138.8	43.	89.6	157.9	43.	89.6
40	0.0	43.	89.6	0.0	43.	89.6
41	137.1	43.	89.6	158.0	42.	87.5
42	0.0	42.	87.5	0.0	42.	87.5
43	136.7	40.	83.3	155.6	40.	83.3
44	0.0	37.	77.1	0.0	40.	83.3
45	132.7	35.	72.9	152.3	40.	83.3
46	0.0	34.	70.8	0.0	37.	77.1
47	133.1	34.	70.8	151.5	36.	75.0
48	0.0	34.	70.8	0.0	32.	66.7
49	131.4	34.	70.8	150.3	32.	66.7
50	0.0	33.	68.8	0.0	28.	58.3
51	133.5	32.	66.7	146.4	27.	56.3
52	0.0	32.	66.7	0.0	26.	54.2

Table 2
Mean Average Weights and Percent Survival in Untreated Control Group I

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
53	0.0	32.	66.7	0.0	25.	52.1
54	134.1	31.	64.6	143.3	23.	47.9
55	0.0	31.	64.6	0.0	23.	47.9
56	134.9	30.	62.5	144.5	22.	45.8
57	0.0	30.	62.5	0.0	22.	45.8
58	133.2	30.	62.5	138.6	22.	45.8
59	0.0	30.	62.5	0.0	20.	41.7
60	134.8	29.	60.4	136.3	20.	41.7
61	0.0	28.	58.3	0.0	18.	37.5
62	0.0	28.	58.3	0.0	18.	37.5
63	133.0	28.	58.3	128.8	17.	35.4
64	0.0	28.	58.3	0.0	15.	31.3
65	0.0	26.	54.2	0.0	15.	31.3
66	139.4	24.	50.0	129.9	15.	31.3
67	0.0	24.	50.0	0.0	15.	31.3
68	138.6	24.	50.0	130.6	14.	29.2
69	0.0	24.	50.0	0.0	12.	25.0
70	0.0	24.	50.0	0.0	11.	22.9
71	138.9	24.	50.0	132.5	11.	22.9
72	0.0	22.	45.8	0.0	11.	22.9
73	140.9	22.	45.8	136.2	10.	20.8
74	0.0	21.	43.8	0.0	8.	16.7
75	141.0	21.	43.8	135.4	8.	16.7
76	0.0	21.	43.8	0.0	8.	16.7
77	141.5	21.	43.8	130.9	7.	14.6
78	0.0	21.	43.8	0.0	7.	14.6
79	141.4	19.	39.6	125.0	7.	14.6
80	0.0	19.	39.6	0.0	5.	10.4
81	0.0	18.	37.5	0.0	3.	6.3
82	140.6	18.	37.5	160.0	1.	2.1
83	0.0	17.	35.4	0.0	1.	2.1
84	142.2	17.	35.4	149.0	1.	2.1
85	0.0	17.	35.4	0.0	1.	2.1
86	142.4	16.	33.3	135.0	1.	2.1
87	0.0	16.	33.3	0.0	0.	0.0
88	140.9	15.	31.3	0.0	0.	0.0
89	0.0	15.	31.3	0.0	0.	0.0
90	142.2	14.	29.2	0.0	0.	0.0
91	0.0	12.	25.0	0.0	0.	0.0
92	0.0	12.	25.0	0.0	0.	0.0
93	124.7	12.	25.0	0.0	0.	0.0
94	0.0	10.	20.8	0.0	0.	0.0
95	131.8	9.	18.8	0.0	0.	0.0
96	0.0	9.	18.8	0.0	0.	0.0
97	130.4	8.	16.7	0.0	0.	0.0
98	0.0	8.	16.7	0.0	0.	0.0
99	127.4	7.	14.6	0.0	0.	0.0
102	138.3	3.	6.3	0.0	0.	0.0
103	0.0	3.	6.3	0.0	0.	0.0
104	131.0	2.	4.2	0.0	0.	0.0
105	0.0	2.	4.2	0.0	0.	0.0
106	142.5	2.	4.2	0.0	0.	0.0

Table 3
Mean Average Weights and Percent Survival in Untreated Control Group II

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
0	89.5	48.	100.0	100.8	48.	100.0
1	91.6	48.	100.0	103.0	48.	100.0
2	101.9	48.	100.0	118.8	48.	100.0
3	104.0	48.	100.0	125.0	48.	100.0
4	111.0	48.	100.0	133.8	48.	100.0
5	112.5	48.	100.0	137.2	48.	100.0
6	117.7	48.	100.0	145.3	48.	100.0
7	122.0	48.	100.0	149.8	48.	100.0
8	126.3	48.	100.0	155.5	48.	100.0
9	129.0	48.	100.0	158.3	48.	100.0
10	130.5	48.	100.0	159.4	48.	100.0
11	132.2	48.	100.0	163.0	48.	100.0
12	0.0	48.	100.0	0.0	48.	100.0
13	137.0	48.	100.0	168.3	48.	100.0
14	137.2	48.	100.0	168.6	48.	100.0
15	138.6	48.	100.0	171.3	48.	100.0
16	142.1	48.	100.0	173.6	48.	100.0
17	143.4	48.	100.0	174.5	48.	100.0
18	145.1	48.	100.0	173.0	48.	100.0
19	144.1	48.	100.0	172.0	48.	100.0
20	147.0	48.	100.0	175.0	48.	100.0
21	146.5	48.	100.0	171.8	47.	97.9
22	146.8	48.	100.0	171.9	47.	97.9
23	147.2	48.	100.0	171.5	47.	97.9
24	147.6	48.	100.0	172.2	46.	95.8
25	147.4	48.	100.0	171.2	46.	95.8
26	145.8	48.	100.0	171.9	46.	95.8
27	146.0	48.	100.0	170.2	46.	95.8
28	147.5	48.	100.0	168.4	46.	95.8
29	148.1	48.	100.0	166.1	45.	93.8
30	148.5	48.	100.0	165.6	43.	89.6
31	148.4	48.	100.0	164.8	40.	83.3
32	148.5	48.	100.0	166.3	39.	81.3
33	149.6	48.	100.0	166.5	39.	81.3
34	148.9	48.	100.0	166.6	39.	81.3
35	149.2	48.	100.0	163.5	39.	81.3
36	150.6	48.	100.0	164.6	38.	79.2
37	150.4	44.	91.7	165.0	38.	79.2
38	150.4	41.	85.4	164.0	38.	79.2
39	152.8	40.	83.3	165.6	37.	77.1
40	0.0	40.	83.3	0.0	35.	72.9
41	151.8	40.	83.3	162.2	35.	72.9
42	0.0	40.	83.3	0.0	34.	70.8
43	150.3	40.	83.3	158.5	33.	68.8
44	0.0	40.	83.3	0.0	33.	68.8
45	149.4	39.	81.3	153.8	32.	66.7
46	0.0	39.	81.3	0.0	32.	66.7
47	147.9	39.	81.3	154.8	30.	62.5
48	0.0	39.	81.3	0.0	27.	56.3
49	148.6	38.	79.2	149.2	25.	52.1
50	0.0	37.	77.1	0.0	23.	47.9
51	147.7	37.	77.1	145.3	23.	47.9
52	0.0	37.	77.1	0.0	22.	45.8

Table 4 - Mean Average Weights and Percent Survival in Untreated Control Group II

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
53	0.0	36.	75.0	0.0	21.	43.8
54	146.6	35.	72.9	133.3	19.	39.6
55	0.0	35.	72.9	0.0	18.	37.5
56	146.0	35.	72.9	137.4	18.	37.5
57	0.0	34.	70.8	0.0	17.	35.4
58	144.1	34.	70.8	133.2	17.	35.4
59	0.0	34.	70.8	0.0	17.	35.4
60	144.5	34.	70.8	132.2	16.	33.3
61	0.0	33.	68.8	0.0	13.	27.1
62	0.0	33.	68.8	0.0	13.	27.1
63	140.5	33.	68.8	126.8	13.	27.1
64	0.0	33.	68.8	0.0	12.	25.0
65	0.0	33.	68.8	0.0	10.	20.8
66	142.4	32.	66.7	125.0	10.	20.8
67	0.0	31.	64.6	0.0	10.	20.8
68	144.0	30.	62.5	122.2	10.	20.8
69	0.0	29.	60.4	0.0	8.	16.7
70	0.0	28.	58.3	0.0	8.	16.7
71	147.6	28.	58.3	124.8	8.	16.7
42	0.0	26.	54.2	0.0	8.	16.7
73	148.5	26.	54.2	114.1	7.	14.6
74	0.0	24.	50.0	0.0	7.	14.6
75	142.2	24.	50.0	131.2	5.	10.4
76	0.0	23.	47.9	0.0	5.	10.4
77	141.9	22.	45.8	131.0	5.	10.4
78	0.0	20.	41.7	0.0	4.	8.3
79	140.4	19.	39.6	125.0	3.	6.3
80	0.0	17.	35.4	0.0	3.	6.3
81	0.0	17.	35.4	0.0	3.	6.3
82	147.3	16.	33.3	126.3	3.	6.3
83	0.0	16.	33.3	0.0	3.	6.3
84	146.9	16.	33.3	119.0	3.	6.3
85	0.0	16.	33.3	0.0	2.	4.2
86	145.1	15.	31.3	132.5	2.	4.2
87	0.0	15.	31.3	0.0	2.	4.2
88	143.9	15.	31.3	140.0	1.	2.1
89	0.0	14.	29.2	0.0	1.	2.1
90	143.7	14.	29.2	126.0	1.	2.1
91	0.0	13.	27.1	0.0	1.	2.1
92	0.0	13.	27.1	0.0	1.	2.1
93	145.1	12.	25.0	0.0	0.	0.0
94	0.0	12.	25.0	0.0	0.	0.0
95	140.5	12.	25.0	0.0	0.	0.0
96	0.0	11.	22.9	0.0	0.	0.0
97	140.2	9.	18.8	0.0	0.	0.0
98	0.0	9.	18.8	0.0	0.	0.0
99	136.8	9.	18.8	0.0	0.	0.0
100	0.0	9.	18.8	0.0	0.	0.0
101	0.0	7.	14.6	0.0	0.	0.0
102	131.0	7.	14.6	0.0	0.	0.0
103	0.0	5.	10.4	0.0	0.	0.0
104	127.4	5.	10.4	0.0	0.	0.0
105	0.0	5.	10.4	0.0	0.	0.0
106	128.5	4.	8.3	0.0	0.	0.0
107	0.0	3.	6.3	0.0	0.	0.0
108	138.3	3.	6.3	0.0	0.	0.0
109	0.0	3.	6.3	0.0	0.	0.0
110	0.0	1.	2.1	0.0	0.	0.0
111	0.0	1.	2.1	0.0	0.	0.0
112	0.0	1.	2.1	0.0	0.	0.0
113	0.0	1.	2.1	0.0	0.	0.0

Table 5
Mean Average Weights and Percent Survival
Following Intratracheal Administration of Vehicle Control

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
0	96.0	90.	100.0	101.0	90.	100.0
1	102.8	90.	100.0	111.2	90.	100.0
2	107.5	90.	100.0	121.3	90.	100.0
3	116.0	90.	100.0	131.2	90.	100.0
4	122.2	90.	100.0	140.5	90.	100.0
5	127.7	90.	100.0	147.3	90.	100.0
6	131.8	90.	100.0	152.7	90.	100.0
7	136.4	90.	100.0	157.8	90.	100.0
8	142.0	90.	100.0	163.7	89.	98.9
9	146.2	90.	100.0	167.6	89.	98.9
10	149.3	90.	100.0	170.7	89.	98.9
11	150.8	89.	98.9	171.7	89.	98.9
12	154.1	89.	98.9	174.9	89.	98.9
13	154.0	88.	97.8	175.3	89.	98.9
14	155.8	88.	97.8	176.0	89.	98.9
15	157.2	88.	97.8	176.6	89.	98.9
16	158.0	88.	97.8	177.1	89.	98.9
17	159.9	87.	96.7	177.1	89.	98.9
18	160.4	86.	95.6	178.4	89.	98.9
19	160.6	85.	94.4	180.0	89.	98.9
20	158.3	85.	94.4	177.5	89.	98.9
21	157.6	85.	94.4	176.8	89.	98.9
22	156.5	85.	94.4	177.1	89.	98.9
23	155.4	85.	94.4	176.1	89.	98.9
24	154.1	85.	94.4	173.7	89.	98.9
25	153.7	85.	94.4	172.6	89.	98.9
26	152.7	84.	93.3	171.7	89.	98.9
27	152.8	84.	93.3	169.9	89.	98.9
28	152.4	84.	93.3	169.9	88.	97.8
29	151.9	84.	93.3	167.6	88.	97.8
30	152.1	84.	93.3	167.4	88.	97.8
31	151.4	84.	93.3	166.4	87.	96.7
32	152.6	84.	93.3	167.0	87.	96.7
33	152.8	84.	93.3	167.0	87.	96.7
34	156.0	84.	93.3	167.2	86.	95.6
35	151.7	87.	96.7	167.8	85.	94.4
36	155.0	84.	93.3	159.6	85.	94.4
37	153.5	84.	93.3	163.8	85.	94.4
38	151.2	84.	93.3	160.6	85.	94.4
39	151.4	82.	91.1	161.8	83.	92.2
40	151.8	82.	91.1	159.9	83.	92.2
41	152.4	82.	91.1	160.6	82.	91.1
42	151.7	82.	91.1	158.9	80.	88.9
43	151.8	80.	88.9	157.2	80.	88.9
44	154.3	78.	86.7	159.5	79.	87.8
45	154.3	78.	86.7	157.9	77.	85.6
46	156.7	76.	84.4	161.7	73.	81.1
47	158.7	74.	82.2	158.7	71.	78.9
48	160.6	74.	82.2	163.5	67.	74.4
49	158.7	73.	81.1	163.1	65.	72.2
50	157.1	72.	80.0	161.0	63	70.0
51	156.1	72.	80.0	161.5	58.	64.4
52	155.7	71.	78.9	160.1	57.	63.3

Table 6
Mean Average Weights and Percent Survival
Following Intratracheal Administration of Vehicle Control

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
53	156.4	70.	77.8	161.5	55.	61.1
54	155.3	69.	76.7	162.2	51.	56.7
55	155.0	69.	76.7	159.9	51.	56.7
56	154.5	68.	75.6	161.2	47.	52.2
57	154.5	67.	74.4	160.9	46.	51.1
58	155.4	67.	74.4	159.6	45.	50.0
59	154.1	67.	74.4	159.9	44.	48.9
60	155.1	65.	72.2	155.1	43.	47.8
61	154.6	64.	71.1	153.9	41.	45.6
62	152.8	64.	71.1	156.1	37.	41.1
63	154.2	62.	68.9	156.2	36.	40.0
64	154.4	62.	68.9	152.6	34.	37.8
65	154.5	60.	66.7	148.6	33.	36.7
66	152.6	60.	66.7	148.0	30.	33.3
67	152.4	60.	66.7	148.3	24.	26.7
68	152.5	57.	63.3	147.1	21.	23.3
69	153.8	56.	62.2	141.8	19.	21.1
70	150.9	55.	61.1	137.4	16.	17.8
71	147.5	53.	58.9	130.4	14.	15.6
72	147.6	50.	55.6	132.3	9.	10.0
73	147.3	48.	53.3	132.0	8.	8.9
74	147.0	47.	52.2	131.8	8.	8.9
75	145.8	46.	51.1	130.1	8.	8.9
76	146.7	42.	46.7	124.5	8.	8.9
77	147.9	41.	45.6	131.0	7.	7.8
78	145.3	38.	42.2	123.8	6.	6.7
79	145.8	38.	42.2	131.8	5.	5.6
80	147.8	36.	40.0	126.3	4.	4.4
81	146.4	35.	38.9	124.5	4.	4.4
82	144.4	33.	36.7	123.8	4.	4.4
83	142.4	33.	36.7	121.3	4.	4.4
84	137.6	33.	36.7	118.0	4.	4.4
85	140.1	31.	34.4	119.3	3.	3.3
86	141.6	28.	31.1	112.3	3.	3.3
87	139.0	27.	30.0	114.7	3.	3.3
88	134.5	24.	26.7	112.5	2.	2.2
89	136.2	22.	24.4	107.5	2.	2.2
90	134.9	20.	22.2	105.0	1.	1.1
91	139.1	17.	18.9	108.0	1.	1.1
92	137.5	17.	18.9	108.0	1.	1.1
93	136.9	14.	15.6	111.0	1.	1.1
94	132.3	12.	13.3	0.0	0.	0.0
95	130.1	11.	12.2	0.0	0.	0.0
96	127.8	10.	11.1	0.0	0.	0.0
97	126.9	7.	7.8	0.0	0.	0.0
98	131.0	6.	6.7	0.0	0.	0.0
99	132.3	6.	6.7	0.0	0.	0.0
100	122.8	6.	6.7	0.0	0.	0.0
101	126.0	4.	4.4	0.0	0.	0.0
102	125.3	3.	3.3	0.0	0.	0.0
103	108.5	2.	2.2	0.0	0.	0.0
104	131.0	1.	1.1	0.0	0.	0.0
105	0.0	1.	1.1	0.0	0.	0.0
106	0.0	0.	0.0	0.0	0.	0.0

Table 7
Mean Average Weights and Percent Survival Following Intratracheal Administration of CD-3 at 0.5 mg/kg Dose Level

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
0	95.2	30.	100.0	108.0	30.	100.0
1	99.3	30.	100.0	115.7	30.	100.0
2	102.5	30.	100.0	124.6	30.	100.0
3	109.3	30.	100.0	135.4	30.	100.0
4	115.2	30.	100.0	143.6	30.	100.0
5	120.3	29.	96.7	150.2	30.	100.0
6	123.4	29.	96.7	156.2	30.	100.0
7	129.2	29.	96.7	163.9	30.	100.0
8	135.7	29.	96.7	169.4	30.	100.0
9	139.8	29.	96.7	174.0	30.	100.0
10	144.5	29.	96.7	177.2	30.	100.0
11	147.5	29.	96.7	179.9	30.	100.0
12	150.2	29.	96.7	182.4	30.	100.0
13	149.8	29.	96.7	182.2	30.	100.0
14	151.1	29.	96.7	181.5	30.	100.0
15	153.9	28.	93.3	179.7	30.	100.0
16	156.1	28.	93.3	178.6	30.	100.0
17	158.0	28.	93.3	179.4	30.	100.0
18	158.9	28.	93.3	180.5	30.	100.0
19	158.3	28.	93.3	180.5	30.	100.0
20	157.4	28.	93.3	178.1	29.	96.7
21	155.9	28.	93.3	176.9	29.	96.7
22	156.1	28.	93.3	177.8	29.	96.7
23	154.1	28.	93.3	175.9	29.	96.7
24	152.7	28.	93.3	174.4	29.	96.7
25	153.0	28.	93.3	173.1	29.	96.7
26	153.4	28.	93.3	172.2	29.	96.7
27	153.0	28.	93.3	169.5	29.	96.7
28	152.8	28.	93.3	167.5	29.	96.7
29	152.9	28.	93.3	167.3	29.	96.7
30	153.0	28.	93.3	163.2	29.	96.7
31	153.0	28.	93.3	164.6	29.	96.7
32	153.9	28.	93.3	163.3	29.	96.7
33	152.9	28.	93.3	162.1	29.	96.7
34	155.8	28.	93.3	164.1	28.	93.3
35	156.1	28.	93.3	164.3	28.	93.3
36	153.8	28.	93.3	163.1	28.	93.3
37	153.2	28.	93.3	164.9	27.	90.0
38	152.9	27.	90.0	162.6	27.	90.0
39	152.9	27.	90.0	160.6	27.	90.0
40	151.3	27.	90.0	157.5	27.	90.0
41	153.8	27.	90.0	159.7	27.	90.0
42	153.2	27.	90.0	158.7	27.	90.0
43	154.0	27.	90.0	160.3	27.	90.0
44	155.1	27.	90.0	161.3	27.	90.0
45	154.3	27.	90.0	160.3	27.	90.0
46	154.7	27.	90.0	160.9	27.	90.0
47	156.0	27.	90.0	164.3	26.	86.7
48	159.0	27.	90.0	164.2	26.	86.7
49	157.2	27.	90.0	166.7	25.	83.3
50	155.3	27.	90.0	163.7	25.	83.3
51	155.0	27.	90.0	158.8	24.	80.0
52	155.7	27.	90.0	156.2	24.	80.0

Table 8
Mean Average Weights and Percent Survival Following Intratracheal Administration of CD-3 at 0.5 mg/kg Dose Level

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
53	155.9	27.	90.0	154.3	24.	80.0
54	154.7	27.	90.0	156.8	21.	70.0
55	155.2	27.	90.0	153.9	19.	63.3
56	154.9	27.	90.0	154.1	17.	56.7
57	155.4	26.	86.7	154.3	16.	53.3
58	155.6	26.	86.7	154.8	14.	46.7
59	155.9	26.	86.7	151.9	14.	46.7
60	155.2	26.	86.7	152.9	13.	43.3
61	156.4	26.	86.7	150.8	12.	40.0
62	155.8	26.	86.7	152.4	11.	36.7
63	155.7	26.	86.7	151.6	10.	33.3
64	155.6	26.	86.7	157.6	9.	30.0
65	155.9	26.	86.7	156.8	9.	30.0
66	155.4	26.	86.7	151.8	9.	30.0
67	150.3	23.	76.7	147.4	9.	30.0
68	150.5	22.	73.3	146.8	8.	26.7
69	150.1	22.	73.3	137.7	7.	23.3
70	153.3	20.	66.7	131.2	6.	20.0
71	150.0	20.	66.7	130.6	5.	16.7
72	150.8	20.	66.7	124.2	5.	16.7
73	149.8	20.	66.7	139.7	3.	10.0
74	149.1	20.	66.7	136.3	3.	10.0
75	148.7	20.	66.7	134.7	3.	10.0
76	149.6	19.	63.3	131.3	3.	10.0
77	150.6	19.	63.3	130.7	3.	10.0
78	148.4	19.	63.3	141.5	2.	6.7
79	149.1	16.	53.3	149.0	2.	6.7
80	152.1	15.	50.0	150.5	2.	6.7
81	151.4	15.	50.0	160.0	2.	6.7
82	151.1	14.	46.7	145.0	1.	3.3
83	148.5	13.	43.3	139.0	1.	3.3
84	148.9	13.	43.3	142.0	1.	3.3
85	143.8	13.	43.3	0.0	0.	0.0
86	143.3	13.	43.3	0.0	0.	0.0
87	141.2	13.	43.3	0.0	0.	0.0
88	141.3	12.	40.0	0.0	0.	0.0
89	140.1	12.	40.0	0.0	0.	0.0
90	138.2	12.	40.0	0.0	0.	0.0
91	138.6	11.	36.7	0.0	0.	0.0
92	141.6	10.	33.3	0.0	0.	0.0
93	137.4	9.	30.0	0.0	0.	0.0
94	137.9	9.	30.0	0.0	0.	0.0
95	144.4	8.	26.7	0.0	0.	0.0
96	149.0	8.	26.7	0.0	0.	0.0
97	144.6	7.	23.3	0.0	0.	0.0
98	140.6	7.	23.3	0.0	0.	0.0
99	128.5	6.	20.0	0.0	0.	0.0
100	127.0	6.	20.0	0.0	0.	0.0
101	125.0	6.	20.0	0.0	0.	0.0
102	126.6	5.	16.7	0.0	0.	0.0
103	125.4	5.	16.7	0.0	0.	0.0
104	123.3	4.	13.3	0.0	0.	0.0
105	0.0	4.	13.3	0.0	0.	0.0
106	0.0	0.	0.0	0.0	0.	0.0

Table 9
Mean Average Weights and Percent Survival Following Intratracheal Administration of CD-3 at 0.25 mg/kg Dose Level

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
0	101.5	30.	100.0	108.2	30.	100.0
1	105.7	30.	100.0	116.5	30.	100.0
2	112.3	30.	100.0	127.6	30.	100.0
3	119.0	30.	100.0	136.7	30.	100.0
4	124.3	30.	100.0	144.2	30.	100.0
5	129.5	30.	100.0	151.3	30.	100.0
6	133.0	30.	100.0	156.9	30.	100.0
7	137.5	30.	100.0	162.7	30.	100.0
8	144.1	30.	100.0	168.2	30.	100.0
9	149.1	30.	100.0	170.1	30.	100.0
10	154.1	29.	96.7	173.1	30.	100.0
11	158.2	29.	96.7	176.3	30.	100.0
12	161.6	29.	96.7	179.1	30.	100.0
13	163.1	29.	96.7	179.4	30.	100.0
14	165.7	29.	96.7	180.1	30.	100.0
15	166.9	27.	90.0	176.8	30.	100.0
16	167.9	27.	90.0	174.0	30.	100.0
17	169.6	27.	90.0	177.4	29.	96.7
18	171.3	27.	90.0	176.7	29.	96.7
19	171.6	27.	90.0	176.7	29.	96.7
20	169.5	27.	90.0	175.7	29.	96.7
21	168.5	27.	90.0	173.8	29.	96.7
22	167.0	26.	86.7	174.2	29.	96.7
23	167.1	24.	80.0	172.2	29.	96.7
24	166.0	24.	80.0	171.8	29.	96.7
25	164.7	24.	80.0	170.5	29.	96.7
26	164.9	24.	80.0	170.4	28.	93.3
27	168.2	23.	76.7	163.8	28.	93.3
28	168.4	23.	76.7	167.1	26.	86.7
29	167.9	23.	76.7	165.2	26.	86.7
30	166.7	23.	76.7	163.8	26.	86.7
31	170.1	22.	73.3	163.3	26.	86.7
32	170.7	22.	73.3	163.9	26.	86.7
33	170.5	22.	73.3	163.0	26.	86.7
34	172.6	22.	73.3	160.9	26.	86.7
35	171.2	22.	73.3	158.0	26.	86.7
36	167.8	22.	73.3	157.5	26.	86.7
37	166.6	21.	70.0	155.3	25.	83.3
38	166.0	21.	70.0	155.2	25.	83.3
39	164.6	21.	70.0	154.0	24.	80.0
40	164.5	21.	70.0	144.2	24.	80.0
41	165.7	21.	70.0	144.9	23.	76.7
42	165.0	21.	70.0	145.8	21.	70.0
43	166.1	21.	70.0	148.6	19.	63.3
44	166.5	21.	70.0	152.2	19.	63.3
45	166.0	21.	70.0	153.7	18.	60.0
46	166.0	21.	70.0	154.2	18.	60.0
47	167.5	21.	70.0	149.9	18.	60.0
48	169.0	21.	70.0	153.4	17.	56.7
49	170.5	20.	66.7	154.8	16.	53.3
50	169.3	20.	66.7	153.7	15.	50.0
51	168.3	20.	66.7	151.2	13.	43.3
52	167.8	20.	66.7	147.5	12.	40.0

Table 10
Mean Average Weights and Percent Survival Following Intratracheal Administration of CD-3 at 0.25 mg/kg Dose Level

WEEK	M AVG WT	N	PCT SURV	F AVG WT	N	PCT SURV
53	167.0	20.	66.7	144.1	12.	40.0
54	166.2	20.	66.7	142.8	11.	36.7
55	165.8	20.	66.7	143.1	10.	33.3
56	164.6	20.	66.7	142.7	9.	30.0
57	163.8	20.	66.7	136.6	8.	26.7
58	163.2	20.	66.7	138.4	7.	23.3
59	161.7	19.	63.3	136.7	7.	23.3
60	161.2	19.	63.3	149.0	5.	16.7
61	162.5	16.	53.3	152.8	4.	13.3
62	163.4	15.	50.0	146.5	4.	13.3
63	164.6	15.	50.0	145.5	4.	13.3
64	163.5	15.	50.0	146.3	4.	13.3
65	164.8	15.	50.0	144.8	4.	13.3
66	162.1	15.	50.0	137.5	4.	13.3
67	164.6	14.	46.7	133.3	4.	13.3
68	157.9	14.	46.7	131.5	4.	13.3
69	158.0	14.	46.7	131.5	4.	13.3
70	156.2	14.	46.7	134.0	3.	10.0
71	156.6	13.	43.3	130.0	3.	10.0
72	155.6	13.	43.3	116.5	2.	6.7
73	154.5	13.	43.3	114.5	2.	6.7
74	154.7	13.	43.3	112.5	2.	6.7
75	153.4	13.	43.3	0.0	0.	0.0
76	151.8	13.	43.3	0.0	0.	0.0
77	157.5	12.	40.0	0.0	0.	0.0
78	155.5	12.	40.0	0.0	0.	0.0
79	154.1	12.	40.0	0.0	0.	0.0
80	149.5	12.	40.0	0.0	0.	0.0
81	149.0	10.	33.3	0.0	0.	0.0
82	151.8	8.	26.7	0.0	0.	0.0
83	148.5	8.	26.7	0.0	0.	0.0
84	148.1	8.	26.7	0.0	0.	0.0
85	145.5	8.	26.7	0.0	0.	0.0
86	141.6	8.	26.7	0.0	0.	0.0
87	139.1	8.	26.7	0.0	0.	0.0
88	137.8	8.	26.7	0.0	0.	0.0
89	129.7	7.	23.3	0.0	0.	0.0
90	130.2	5.	16.7	0.0	0.	0.0
91	127.0	5.	16.7	0.0	0.	0.0
92	124.0	5.	16.7	0.0	0.	0.0
93	124.5	4.	13.3	0.0	0.	0.0
94	126.7	3.	10.0	0.0	0.	0.0
95	117.0	3.	10.0	0.0	0.	0.0
96	129.0	2.	6.7	0.0	0.	0.0
97	125.5	2.	6.7	0.0	0.	0.0
98	121.5	2.	6.7	0.0	0.	0.0
99	131.0	1.	3.3	0.0	0.	0.0
100	139.0	1.	3.3	0.0	0.	0.0
101	132.0	1.	3.3	0.0	0.	0.0
102	0.0	0.	0.0	0.0	0.	0.0

SUMMARY OF GROSS PATHOLOGY INCIDENCE TABLE 11

CD-3 INTRATRACHEAL

	UUC		UUB		VC		0.5 mg		0.25 mg	
	M	F	M	F	M	F	M	F	M	F
SPLEEN	43	44	48	44	90	89	29	30	30	30
Enlarged	1	7	1	12	11	20	5	8	1	12
Atrophic	0	0	0	0	0	0	1	0	0	0
INTESTINE	48	44	48	44	90	89	29	30	30	28
Bloated	3	2	1	3	4	3	0	0	0	1
Enlarged	0	0	0	0	0	0	0	1	0	0
Hemorrhagic	0	1	5	2	4	2	0	1	1	1
Atrophy	0	0	0	0	0	0	0	0	0	1
Abscess	0	0	0	0	1	0	0	0	1	0
Adhesion	0	0	0	0	0	0	0	0	1	0
KIDNEY	48	44	48	44	90	89	29	30	30	30
Pale	6	7	5	13	21	16	11	7	8	12
Granular	6	12	10	11	16	37	8	11	9	15
Mass	0	0	2	0	1	0	0	0	0	3
Discolored	0	0	0	0	0	0	1	0	0	0
Enlarged	0	3	1	4	0	1	0	0	0	3
Mottled	0	0	0	0	0	0	1	0	2	0
Necrotic area	0	0	0	0	0	0	0	1	0	0
LIVER	48	44	48	44	90	89	29	30	30	30
Pale	9	3	6	0	15	9	1	5	3	1
Mottled	1	6	0	3	12	13	8	5	5	4
Granular	0	1	2	0	5	0	2	0	0	0
Lobular pattern	10	9	9	14	16	28	0	8	1	8
Dark	0	0	0	0	0	0	0	0	1	8
Enlarged	1	3	2	3	3	6	0	1	0	3
Cystic	2	9	4	7	9	11	3	5	3	2
Congested	2	1	6	0	4	5	0	0	2	0
Discolored	0	1	0	1	3	0	0	0	0	4
Blood clot - hemorrhage	0	0	0	0	1	0	1	0	0	0
STOMACH	48	44	48	44	90	89	29	30	30	30
Ulcers	0	0	0	0	1	6	0	0	0	1
ADRENALS	48	44	48	44	90	89	29	30	30	30
Pale	0	2	2	1	3	7	2	1	2	1
Enlarged	0	1	0	0	3	1	1	0	1	2
Discolored	0	0	0	0	0	0	0	0	0	1
SKIN	48	44	48	44	90	89	29	30	30	30
Subcutaneous edema	2	0	5	2	7	7	0	0	2	0

SUMMARY OF GROSS PATHOLOGY INCIDENCE TABLE 12

CD-3 INTRATRACHEAL

	UUC		UUB		VC		0.5 mg		0.25 mg	
	M	F	M	F	M	F	M	F	M	F
LUNG	47	41	48	44	90	88	28	30	30	30
Congested	18	20	17	12	45	43	15	17	14	11
Hemorrhagic	9	5	9	5	38	21	13	6	9	6
Enlarged - swollen	0	0	0	0	0	0	0	0	0	2
Mottled	2	0	5	5	9	11	3	1	1	3
Pale	0	1	0	6	1	2	0	0	1	0
Emphysema	9	3	6	7	12	26	2	3	7	4
Exudate	0	0	0	0	0	0	0	1	0	0
BLADDER	48	44	48	44	90	89	29	30	30	30
Filled	8	0	5	3	34	4	14	3	9	2
LYMPH NODES	48	44	48	44	90	89	29	30	30	30
Brachial enlarged	1	1	3	3	5	2	0	0	1	0
Inguinal enlarged	24	18	24	15	53	49	11	13	7	17
Cervical enlarged	5	7	11	6	18	23	6	11	9	11
Axillary enlarged	0	0	0	0	0	0	0	0	0	2
ABDOMINAL CAVITY	48	44	48	44	90	89	29	30	30	30
Fluid	1	3	13	7	3	8	0	0	2	6
THORACIC CAVITY	48	44	48	44	90	89	29	30	30	30
Fluid	6	4	1	2	10	11	0	0	2	1
OVARIES	0	44	0	44	0	87	0	30	0	30
Cystic	0	0	0	3	0	5	0	0	0	1
TESTIS	48	0	48	0	90	0	29	0	30	0
Abscess	0	0	1	0	2	0	0	0	1	0
EPIDIDYMIS	48	0	48	0	90	0	29	0	30	0
Abscess	0	0	0	0	1	0	0	0	1	0
THYROID	48	44	48	44	90	89	29	30	30	30
Enlarged	0	0	0	1	0	8	0	1	0	7

NEOPLASM SUMMARY TABLE 13

CD-3 INTRATRACHEAL

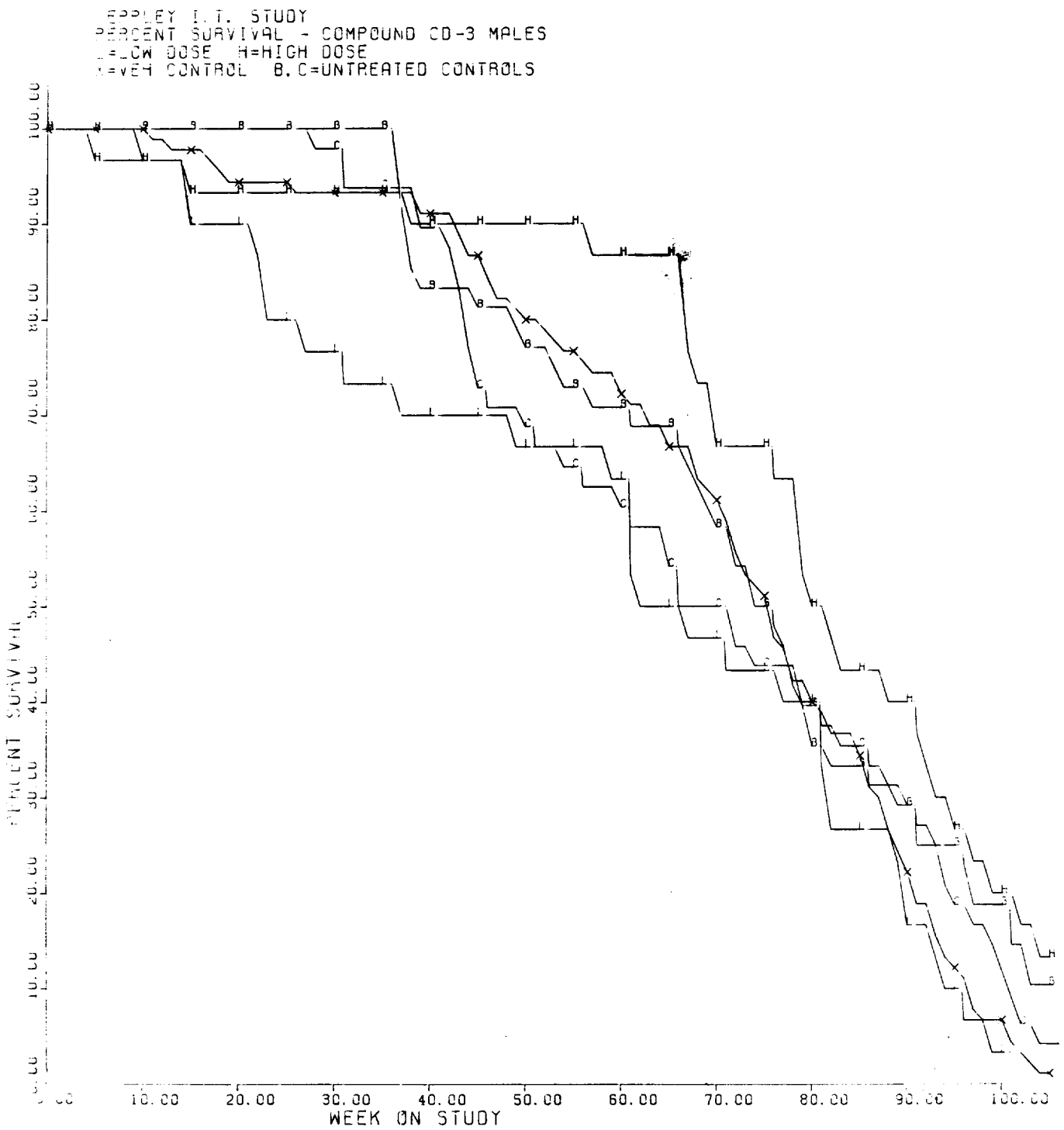
Group:	UCC		UUB		VC		0.5 mg		0.25 mg	
	M	F	M	F	M	F	M	F	M	F
LARYNX	44	36	46	39	43	59	28	17	26	22
LUNGS	47	42	46	44	82	77	27	24	26	28
Carcinoma metastatic	0	0	1	0	0	0	0	0	0	0
Bronchiolar adenoma	0	0	0	0	0	0	0	0	1	0
STOMACH	47	41	46	41	1	5	0	0	0	1
Papilloma	0	0	1	1	0	3	0	0	0	0
INTESTINES	46	41	45	39	2	1	0	0	0	0
Adenocarcinoma	1	0	0	0	0	0	0	0	0	0
LIVER	44	43	45	40	57	61	12	18	8	25
Lymphoma metastatic	0	0	1	0	0	0	0	0	0	0
Sarcoma metastatic	0	0	1	0	0	0	0	0	0	0
Cholangioma	2	0	2	1	1	0	0	0	0	0
Hemangioma	0	0	0	0	1	0	0	0	0	0
Hemangiosarcoma	1	0	0	0	0	0	0	0	0	0
KIDNEY	46	43	46	43	56	70	13	21	13	26
Sarcoma - metastatic	0	0	1	0	0	0	0	0	0	0
Carcinoma - metastatic	0	0	1	0	0	0	0	0	0	0
SPLEEN	39	40	41	41	12	36	4	14	2	15
Lymphoma	0	0	0	1	0	0	0	0	0	0
Lymphoma - metastatic	0	0	1	0	1	0	0	0	0	0
Sarcoma - metastatic	0	0	1	0	1	0	0	0	0	0
Hemangiosarcoma	1	0	0	0	0	0	0	0	0	0
LYMPH NODES	2	5	8	3	21	38	3	9	3	5
Lymphoma	0	0	2	0	3	0	0	0	1	0
BONE MARROW	47	38	45	40	37	21	2	0	2	6
THYMUS	0	0	1	0	0	0	0	0	0	1
Lymphoma - metastatic	0	0	1	0	0	0	0	0	0	0
PANCREAS	30	28	25	23	0	3	0	0	0	0
Islet cell adenoma	0	0	1	0	0	0	0	0	0	0
ADRENAL GLAND	45	41	45	42	35	47	6	8	5	19
Cortical adenoma	10	2	5	1	5	3	0	0	2	0
Cortical carcinoma	0	0	2	0	0	0	0	0	0	0
Pheochromocytoma	0	0	1	0	0	0	0	0	0	0
Lymphoma - metastatic	0	0	0	0	1	0	0	0	0	0

NEOPLASM SUMMARY TABLE 14

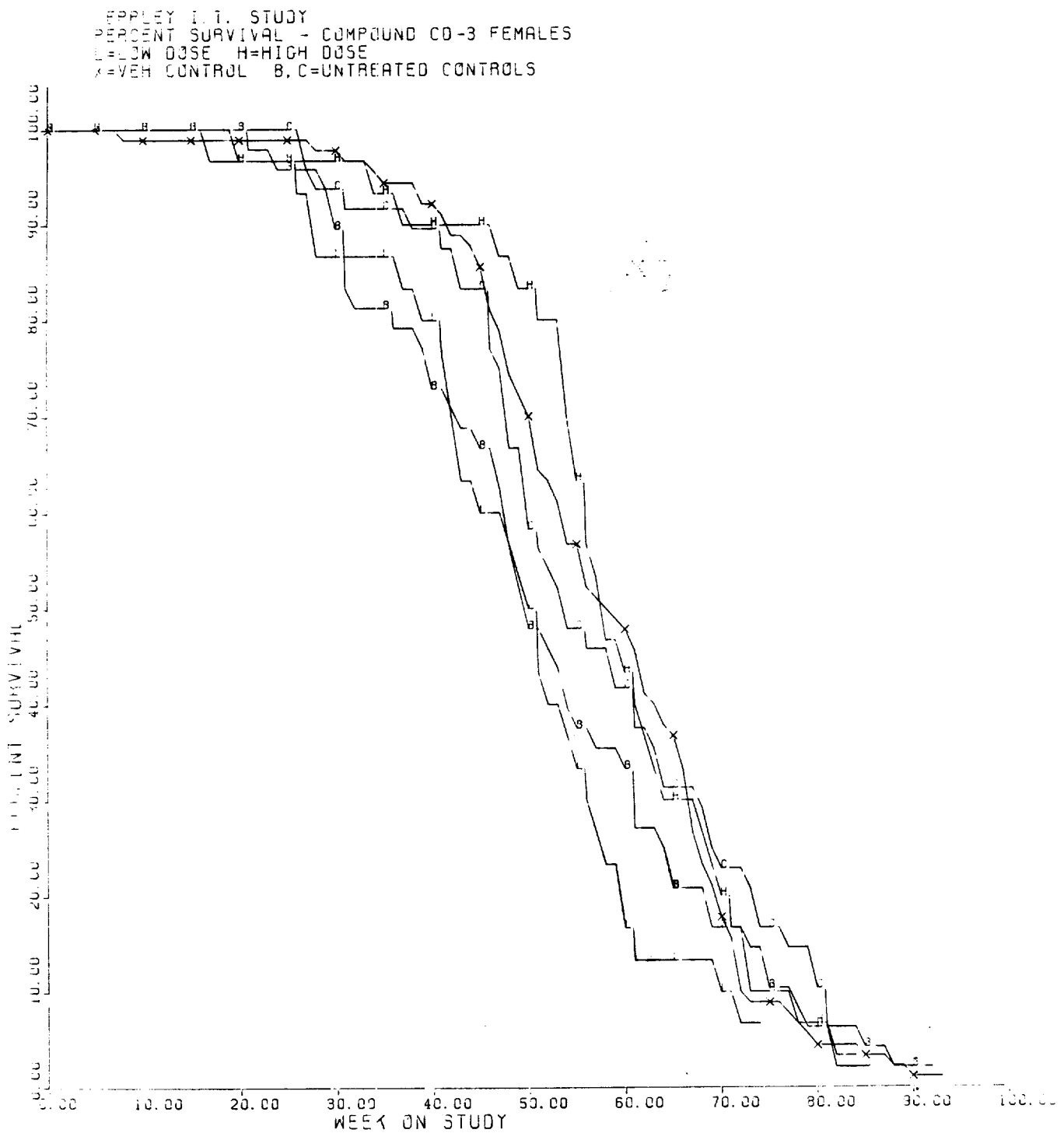
CD-3 INTRATRACHEAL

Group:	UUC		UUB		VC		0.5 mg		0.25 mg	
	M	F	M	F	M	F	M	F	M	F
OVARY	0	31	0	25	0	3	0	0	0	1
Granulosa cell tumor	0	0	0	1	0	0	0	0	0	0
THYROID GLAND	1	6	0	0	27	42	4	8	6	14
Adenoma	0	0	0	0	1	0	0	0	0	0
PARATHYROID GLAND	0	0	0	0	1	9	1	1	0	5
SKIN	1	0	0	1	0	2	2	0	0	0
Melanoma	1	0	0	0	0	0	0	0	0	0
THORACIC WALL	0	0	1	0	0	0	0	0	0	0
Hemangioma	0	0	1	0	0	0	0	0	0	0
LEFT HIND LIMB	0	0	1	0	0	0	0	0	0	0
Sarcoma	0	0	1	0	0	0	0	0	0	0
BONE	0	0	0	0	1	2	0	0	0	1

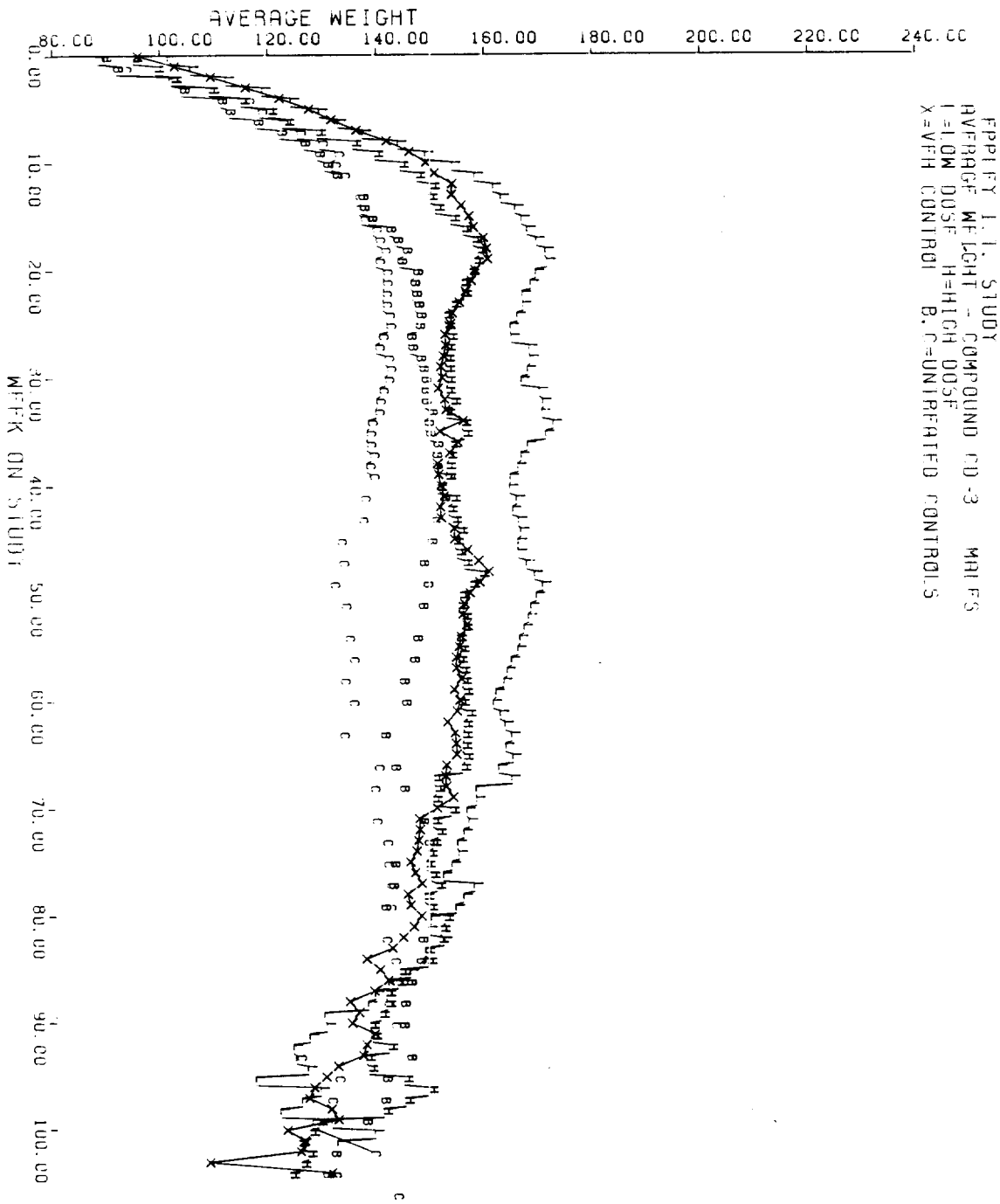
Graph 1


PERCENT SURVIVAL - COMPOUND CD-3 MALES
H=HIGH DOSE
B,C=UNTREATED CONTROLS
PERCENT SURVIVAL
WEEK ON STUDY
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00

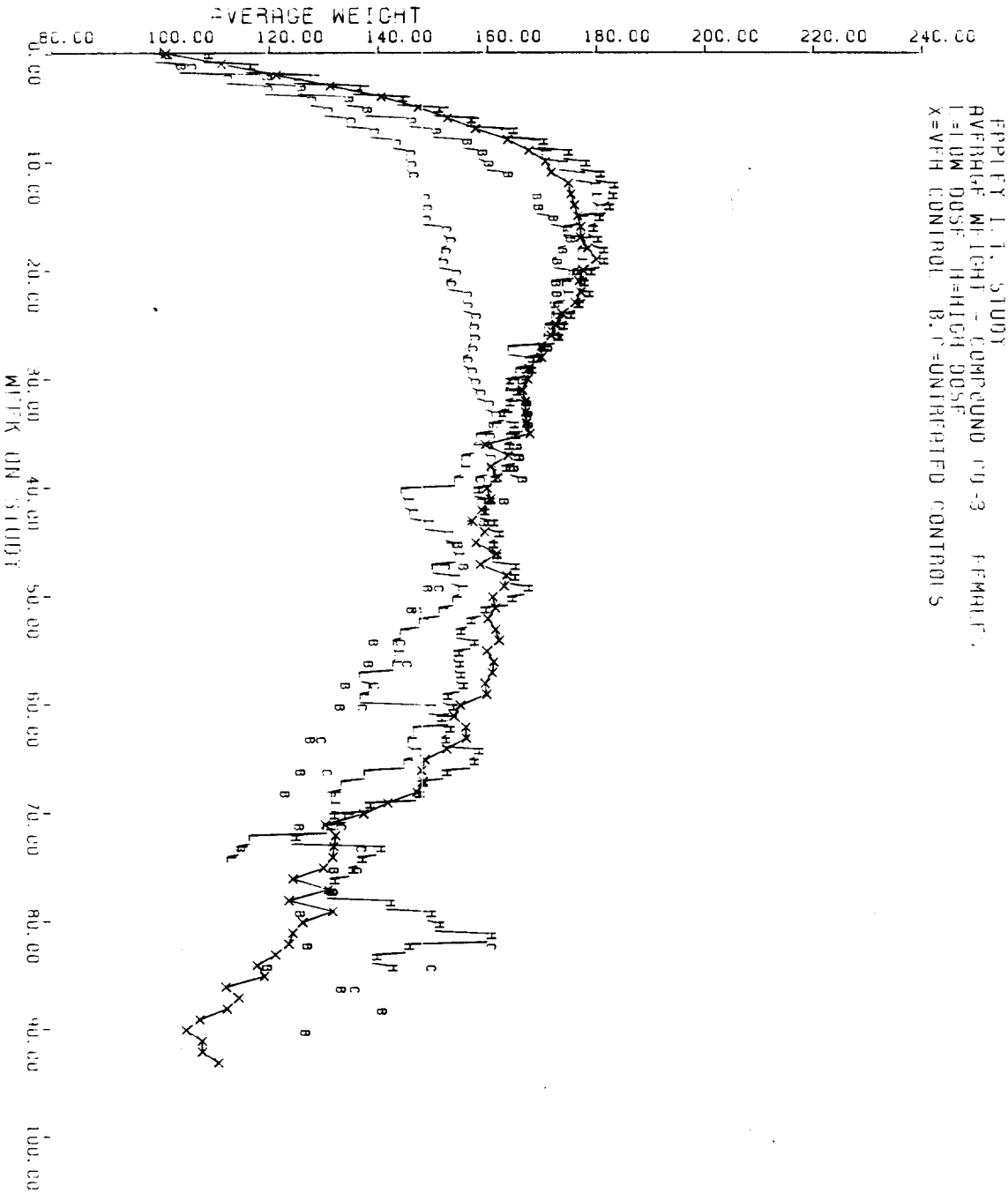
Graph 2


EPPLEY I.T. STUDY
PERCENT SURVIVAL - COMPOUND CO-3 FEMALES
L=LOW DOSE H=HIGH DOSE
X=VEH CONTROL B,C=UNTREATED CONTROLS
WEEK ON STUDY

Graph 3


FIPLEY I.I. STUDY
AVERAGE WEIGHT - COMPOUND CD-3 MALES
L=LOW DOSE H=HIGH DOSE
X=VEH CONTROL B,C=UNTREATED CONTROLS
AVERAGE WEIGHT
80.00
100.00
120.00
140.00
160.00
180.00
200.00
220.00
240.00
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00
WEEK ON STUDY

Graph 4


EPPLEY I.T. STUDY
AVERAGE WEIGHT - COMPOUND CU-3 FEMALE
L=LOW DOSE H=HIGH DOSE
X=VEH CONTROL B,C=UNTREATED CONTROLS
AVERAGE WEIGHT
80.00
100.00
120.00
140.00
160.00
180.00
200.00
220.00
240.00
WEEK ON STUDY
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00

SUMMARY OF HISTOPATHOLOGY INCIDENCE TABLE

CD-3 INTRATRACHEAL

Group:	UUC		UUB		VC		0.5 mg		0.25 mg	
	M	F	M	F	M	F	M	F	M	F
LUNGS	47	42	46	44	82	77	27	24	26	28
Congestion	36	21	24	18	59	52	10	16	9	18
Inflammation	9	17	17	16	13	31	4	11	4	11
Bronchiolar epith. hyperpl.	6	6	11	5	9	6	2	2	2	5
Alveolar cell metaplasia	7	4	6	7	17	10	15[1,2,3]	1	10[1,2]	2
Atelectasis	0	3	0	0	0	0	0	1	0	0
Squamous metaplasia	0	0	0	0	3	3	2	0	1	0
Hemorrhage	0	0	0	0	1	2	0	1	1	3
Epithelial desquamation	0	0	0	0	0	3	0	0	0	1
Inflammatory exudate	0	0	0	0	0	0	0	1	0	1
Bronchiolar epith. atrophy	0	0	0	0	0	0	0	0	1	0
TRACHEA	42	40	44	40	25	45	6	15	9	21
Inflammation	0	0	3	3	0	2	0	0	0	2
Desquamation	9	6	14	6	2	4	0	0	2	5
Epithelial cell atrophy	0	0	1	0	3	2	0	0	2	6
Hyperplasia	0	1	0	6	4	2[1,2]	0	0	3[1,2]	2[2]
Detritus in lumen	0	0	0	0	0	0	0	0	0	1
ADRENAL GLAND	45	41	45	42	35	47	6	8	5	9
Amyloidosis	6	27	11	27	3	3	1	0	2	5
Decomposition	1	1	5	1	3	0	0	0	0	0
Thrombosis	1	1	1	0	0	0	0	0	0	0
Congestion	2	5	3	1	0	0	0	0	0	0
Hemorrhage	1	0	0	0	7	10	1	0	0	1
Infarct	0	0	0	0	0	1	0	0	0	0
Lipid accumulation	0	0	0	0	1	0	0	0	0	0
Degeneration	0	0	0	0	3	1	0	0	1	6
LARYNX	44	36	46	39	43	59	28	17	26	22
Inflammation	2	4	3	4	1	0	1	1	0	2
Epithelial hyperplasia	0	0	2	1	0	6	1	3[1,2]	1	3[1]
Degeneration	0	0	0	0	0	1	0	0	3	2
Desquamation	0	0	0	0	1	0	0	0	0	3
URINARY BLADDER	43	36	40	30	1	0	1	1	1	0
Epithelial degeneration	16	6	14	4	0	0	0	0	0	0
Decomposition	13	6	6	4	0	0	0	0	0	0
Epithelial hyperplasia	0	0	0	0	0	0	1	1	0	0
INTESTINES	46	41	45	39	2	1	0	0	0	0
Inflammation	3	2	1	3	0	0	0	0	0	0
Lymphoid hyperplasia	0	0	0	1	0	0	0	0	0	0
Epithelial degeneration	0	1	2	3	0	0	0	0	0	0
Decomposition	2	18	13	7	0	0	0	0	0	0
Parasites in lumen	0	1	0	0	0	0	0	0	0	0
Atypia epithelial cells	0	1	0	0	0	0	0	0	0	0

SUMMARY OF HISTOPATHOLOGY INCIDENCE TABLE 16

CD-3 INTRATRACHEAL

Group:	UUC		UUB		VC		0.5 mg		0.25 mg	
	M	F	M	F	M	F	M	F	M	F
BONE	0	0	0	0	1	2	0	0	0	1
TESTES	44	0	43	0	4	0	0	0	4	0
Degeneration	3	0	9	0	2	0	0	0	0	0
Inflammation	0	0	0	0	1	0	0	0	4	0
THYROID	1	6	0	0	27	42	4	8	6	14
Degeneration	0	0	0	0	1	2	0	0	3	1
Inflammation	0	0	0	0	0	1	0	0	0	6
Amyloidosis	0	1	0	0	0	0	0	0	0	0
Enlarged	0	0	0	0	0	2	0	0	1	0
SKIN	1	0	0	1	0	2	2	0	0	0
BONE MARROW	47	38	45	40	37	21	2	0	2	6
Degeneration	0	0	0	1	5	0	0	0	0	0
Hemorrhage	2	1	0	0	0	0	0	0	0	0
Decomposition	0	0	1	0	0	0	0	0	0	0
LIVER	44	43	45	40	57	61	12	18	8	25
Congestion	29	28	35	23	33	44	3	9	4	14
Bile duct proliferation	4	1	2	5	0	3	0	1	0	3
Bile duct dilation	0	0	2	1	0	3	0	0	0	2
Amyloidosis	2	26	5	10	5	8	4	2	1	5
Bile stasis	0	0	0	1	0	0	0	0	0	0
Decomposition	0	1	6	2	2	4	1	0	1	0
Edema	0	0	0	1	0	0	0	0	1	1
Giant cells	8	1	2	3	3	1	0	0	0	0
Liver cell degeneration	1	0	0	0	14	12	0	1	2	8
Cyst	0	6	0	3	3	1	1	0	0	0
Lymphoid cell infiltrates	0	0	0	0	1	4	1	1	2	2
Fibrosis	0	0	0	0	0	10	0	5	1	0
Hemorrhage	0	0	0	0	2	4	2	1	0	0
Vascular wall alteration	0	0	0	1	0	0	0	0	0	1
EPIDIDYMIS	3	0	16	0	1	0	0	0	0	0
Retention	0	0	1	0	0	0	0	0	0	0
Inflammation	0	0	1	0	1	0	0	0	0	0
Decomposition	1	0	3	0	0	0	0	0	0	0
STOMACH	47	41	46	41	1	5	0	0	0	1
Inflammation	0	1	1	0	0	0	0	0	0	0
Degeneration	0	1	1	0	0	0	0	0	0	0
Decomposition	2	6	7	4	0	0	0	0	0	0
Hemorrhage	0	1	1	0	0	0	0	0	0	0

SUMMARY OF HISTOPATHOLOGY INCIDENCE TABLE 17

CD-3 INTRATRACHEAL

Group:	UUC		UUB		VC		0.5 mg		0.25 mg	
	M	F	M	F	M	F	M	F	M	F
PANCREAS	30	28	25	23	3	1	1	0	0	0
Inflammation	6	0	4	5	0	0	0	0	0	0
Decomposition	6	2	6	2	0	0	0	0	0	0
OVARY	0	31	0	25	0	3	0	0	0	1
Cyst	0	1	0	5	0	0	0	0	0	1
Decomposition	0	2	0	0	0	0	0	0	0	0
KIDNEY	46	43	46	43	56	70	13	21	13	26
Amyloidosis	4	12	6	14	1	0	0	0	0	1
Nephropathy	14	28	19	25	27	18	6	6	9	5
Tubular alteration	30	21	26	17	0	4	0	3	0	2
Congestion	31	10	19	12	7	10	4	1	0	0
Acute pyelonephritis	0	0	2	1	3	0	2	0	0	5
Chronic pyelonephritis	0	0	0	0	3	3	0	0	1	3
Decomposition	0	1	3	2	0	0	1	0	0	0
Inflammation	1	1	0	1	9	40	1	16	3	15
Ischemia	0	0	0	0	0	1	0	0	0	1
Embolism	0	0	0	0	0	0	0	1	0	0
Vascular wall alteration	0	0	0	0	0	0	0	0	0	2
UTERUS	0	36	0	36	0	4	0	0	0	0
Squamous metaplasia	0	2	0	3	0	1	0	0	0	0
Endometrial hyperplasia	0	1	0	3	0	1	0	0	0	0
Inflammation	0	0	0	3	0	0	0	0	0	0
Decomposition	0	2	0	0	0	0	0	0	0	0
SPLEEN	39	40	41	41	12	36	4	14	2	15
Amyloidosis	6	24	6	21	1	1	0	0	0	4
Congestion	6	5	2	3	1	5	0	1	0	2
Decomposition	3	3	2	3	0	0	0	0	0	0
Fibrosis	0	0	0	0	2	0	0	0	0	0
Infarct	0	0	0	0	2	1	0	0	0	0
Hemorrhage	0	0	0	0	1	0	0	0	0	0
Lymphatic hyperplasia	0	1	0	0	0	0	0	0	0	0
Enlarged	0	0	0	0	0	1	0	0	0	0
SALIVARY GLAND	5	0	1	4	0	0	0	1	0	0
Inflammation	0	0	0	1	0	0	0	1	0	0
Hyperplasia	5	0	1	3	0	0	0	0	0	0
LYMPH NODES	2	5	8	3	21	38	3	9	3	5
Hyperplasia	2	3	6	3	1	2	0	0	0	2
Inflammation	0	1	0	0	1	5	0	0	0	0

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Evaluation of the Carcinogenic Potential in Hamsters Following Feeding of CD-3

Acc. No. 905401

Toxicology Section

Milan S. Vlaovic, D.V.M., Ph.D.

November 10, 1981

HEALTH, SAFETY AND HUMAN FACTORS LABORATORY
EASTMAN KODAK COMPANY, Rochester, N.Y. 14650

NOT FOR PUBLICATION

The carcinogenic potential of 4-amino-3-methyl-N-ethyl-N-(β-methylsulfonamidoethyl)anilinesesguisulfate monohydrate (CD-3) (Acc. No. 905401) was evaluated by feeding to hamsters. The testing facility was the Eppley Institute for Research in Cancer, University of Nebraska Medical Center at Omaha, Nebraska, under the sponsorship of Eastman Kodak Company. The feeding study was conducted in compliance with the protocol accepted by Eastman Kodak Company on June 21, 1973. (Addendum)

TEST MATERIAL

A representative sample of the test compound was submitted to the Eppley Institute by Eastman Kodak Company. It was accompanied by analytical data, acute toxicity data and a health hazard summary that was originated in the Health, Safety, and Human Factors Laboratory.

ANIMALS

Male and female random bred Syrian golden hamsters from Lakeview Hamsters, New Field, New Jersey, were used for this study.

HOUSING

One control and three test groups containing 35 males and 35 females each were used. The animals were housed five per cage by sex. The four groups of animals were housed in plastic cages under optimum hygienic conditions in temperature and humidity controlled rooms. A corn cob bedding (Bed-O'-Cobs) supplied by SASCO, Inc., 612 N. 16th Street, Omaha, Nebraska, was used. Water was available ad libitum throughout the experimental period.

TREATMENT

The control group of hamsters received a basal diet of Wayne Lab Blox pellets (Allied Mills, Inc., Chicago, Ill.) throughout the two-year experimental period. The three test groups received the CD-3 compound at dosage levels of 0.01% (low), 0.02% (intermediate) or 0.04% (high). An appropriate amount of CD-3 to achieve the designated dosages was blended with Wayne Lab Blox in the meal form and was fed to the hamsters 7 days per week for up to two years. The meal and pelleted forms of the Wayne Lab Blox are identical in composition.

SURVIVAL

Mortality, physical appearance and behavior were monitored daily. The survival of the hamsters in control and test groups was excellent. Greater than 50% of the control and CD-3 treated males survived to 80 experimental weeks. The survival of control and CD-3 treated females was 50% or greater at 59 experimental weeks. In addition, the 50% survival period was twenty weeks longer in males than in females. The survival rates for control and test groups in the feeding study were comparable and are presented in Graphs 7 and 8 and in Tables 1-16.

OBSERVATIONS

Body weights were monitored weekly. The male hamsters peaked in weight between the 30th and 35th experimental weeks and maintained similar weight throughout the experimental period. No significant differences in body weights among the groups of male hamsters were

apparent. The female hamsters attained their highest weight near 30 weeks and sustained this weight for approximately four months. During the remainder of the experiment, female hamsters in control and treated groups gradually decreased in weight. Body weights are presented on Graphs 1 and 2 and are reported in Tables 1-16.

Food consumption was monitored weekly. Male and female hamsters consumed approximately the same quantity of food whether it was basal diet alone or diet supplemented with the CD-3 test compound (Graphs 3 and 4 and Tables 1-16). Average compound intake for each hamster per week is presented on Graphs 5 and 6, and summary Tables 1-16. All raw data are presented in the addendum.

PATHOLOGY

Complete necropsies were performed on all hamsters found dead or sacrificed. Summary of gross pathology is presented on Tables 17-19. All tumors and other pathological lesions were examined grossly and histopathologically in addition to sections of thyroid, heart, lung, liver, spleen, stomach, small and large intestine, pancreas, kidney, urinary bladder, adrenal, gonad, lymph node, bone marrow and brain. All tissue sections were preserved in 10% buffered formalin and stained with H and E for microscopic evaluation. Table 20 presents a summary of tumors diagnosed in control and CD-3 fed hamsters. No treatment related tumors were observed.

Tables 21-24 present a summary of histopathological findings exclusive of tumors. No treatment related changes were observed.

Table 1
Mean Average Weight, Feed and Compound
Consumption and Survival in Male Control Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
0	35.	0	105.2	487.0	13.9	0.0	0.0
1	35.	0	105.1	263.0	7.5	0.0	0.0
2	35.	0	112.1	314.0	9.0	0.0	0.0
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	124.3	0.0	0.0	0.0	0.0
5	35.	0	131.0	337.0	9.6	0.0	0.0
6	35.	0	133.2	340.0	9.7	0.0	0.0
7	35.	0	137.0	398.0	11.4	0.0	0.0
8	35.	0	137.3	371.0	10.6	0.0	0.0
9	35.	0	129.7	272.0	7.8	0.0	0.0
10	35.	0	131.5	380.0	10.9	0.0	0.0
11	35.	0	135.0	365.0	10.4	0.0	0.0
12	35.	0	136.7	308.0	8.8	0.0	0.0
13	35.	0	136.7	0.0	0.0	0.0	0.0
14	35.	0	137.4	323.0	9.2	0.0	0.0
15	35.	0	139.2	488.0	13.9	0.0	0.0
16	35.	0	135.8	387.0	11.1	0.0	0.0
17	35.	0	140.4	454.0	13.0	0.0	0.0
18	35.	0	141.7	417.0	11.9	0.0	0.0
19	35.	0	140.0	523.0	14.9	0.0	0.0
20	35.	0	142.9	441.0	12.6	0.0	0.0
21	35.	0	144.3	407.0	11.6	0.0	0.0
22	35.	0	147.6	408.0	11.7	0.0	0.0
23	35.	0	149.3	376.0	10.7	0.0	0.0
24	0.	0	0.0	0.0	0.0	0.0	0.0
25	35.	0	148.7	282.0	8.1	0.0	0.0
26	35.	0	147.4	373.0	10.7	0.0	0.0
27	35.	0	147.9	383.0	10.9	0.0	0.0
28	35.	0	146.5	288.0	8.2	0.0	0.0
29	35.	0	159.9	163.0	4.7	0.0	0.0
30	35.	0	147.0	366.0	10.5	0.0	0.0
31	35.	0	144.0	318.0	9.1	0.0	0.0
32	35.	0	146.3	480.0	13.7	0.0	0.0
33	35.	0	151.0	379.0	10.8	0.0	0.0
34	35.	0	150.9	305.0	8.7	0.0	0.0
35	35.	0	150.6	435.0	12.4	0.0	0.0
36	35.	0	150.3	319.0	9.1	0.0	0.0
37	35.	0	150.3	347.0	9.9	0.0	0.0
38	35.	0	147.6	422.0	12.1	0.0	0.0
39	34.	1	147.6	379.0	11.0	0.0	0.0
40	34.	0	145.3	422.0	12.4	0.0	0.0
41	34.	0	144.5	410.0	12.1	0.0	0.0
42	34.	0	143.1	425.0	12.5	0.0	0.0
43	34.	0	141.4	349.0	10.3	0.0	0.0
44	34.	0	140.2	387.0	11.4	0.0	0.0
45	33.	1	140.5	432.0	12.9	0.0	0.0
46	33.	0	141.2	351.0	10.6	0.0	0.0
47	33.	0	141.1	375.0	11.4	0.0	0.0
48	32.	1	141.0	515.0	15.8	0.0	0.0
49	30.	2	138.2	386.0	12.5	0.0	0.0
50	30.	0	140.6	399.0	13.3	0.0	0.0
51	30.	0	139.7	386.0	12.9	0.0	0.0
52	30.	0	140.4	412.0	13.7	0.0	0.0

Table 2
Mean Average Weight, Feed and Compound
Consumption and Survival in Male Control Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
53	30.	0	141.1	337.0	11.2	0.0	0.0
54	30.	0	140.7	319.0	10.6	0.0	0.0
55	30.	0	141.6	274.0	9.1	0.0	0.0
56	30.	0	140.3	275.0	9.2	0.0	0.0
57	30.	0	141.5	337.0	11.2	0.0	0.0
58	30.	0	140.8	298.0	9.9	0.0	0.0
59	30.	0	140.5	326.0	10.9	0.0	0.0
60	30.	0	140.5	381.0	12.7	0.0	0.0
61	30.	0	140.2	360.0	12.0	0.0	0.0
62	30.	0	141.1	387.0	12.9	0.0	0.0
63	30.	0	139.3	371.0	12.4	0.0	0.0
64	30.	0	139.3	366.0	12.2	0.0	0.0
65	30.	0	137.8	406.0	13.5	0.0	0.0
66	29.	1	141.9	442.0	15.0	0.0	0.0
67	29.	0	141.7	545.0	18.8	0.0	0.0
68	29.	0	129.3	366.0	12.6	0.0	0.0
69	28.	1	138.0	370.0	13.2	0.0	0.0
70	27.	1	135.8	308.0	11.4	0.0	0.0
71	26.	1	135.4	265.0	10.2	0.0	0.0
72	26.	0	133.9	274.0	10.5	0.0	0.0
73	26.	0	134.7	270.0	10.4	0.0	0.0
74	26.	0	131.3	344.0	13.2	0.0	0.0
75	25.	1	136.3	273.0	10.7	0.0	0.0
76	24.	1	139.0	169.0	6.9	0.0	0.0
77	24.	0	137.6	164.0	6.8	0.0	0.0
78	23.	1	140.7	216.0	9.2	0.0	0.0
79	23.	0	137.6	183.0	8.0	0.0	0.0
80	23.	0	136.8	225.0	9.8	0.0	0.0
81	23.	0	138.0	199.0	8.7	0.0	0.0
82	22.	1	138.3	134.0	6.0	0.0	0.0
83	21.	1	133.2	167.0	7.8	0.0	0.0
84	19.	2	135.1	173.0	8.6	0.0	0.0
85	18.	1	135.1	177.0	9.6	0.0	0.0
86	18.	0	136.2	192.0	10.7	0.0	0.0
87	18.	0	138.0	164.0	9.1	0.0	0.0
88	17.	1	136.3	214.0	12.2	0.0	0.0
89	17.	0	139.9	177.0	10.4	0.0	0.0
90	16.	1	138.9	206.0	12.5	0.0	0.0
91	16.	0	131.5	189.0	11.8	0.0	0.0
92	16.	0	132.5	137.0	8.6	0.0	0.0
93	14.	2	132.1	140.0	9.3	0.0	0.0
94	14.	0	132.9	141.0	10.1	0.0	0.0
95	12.	2	140.8	120.0	9.2	0.0	0.0
96	12.	0	139.0	119.0	9.9	0.0	0.0
97	12.	0	144.3	111.0	9.3	0.0	0.0
98	11.	1	141.6	138.0	12.0	0.0	0.0
99	10.	1	142.6	82.0	7.8	0.0	0.0
100	10.	0	141.7	105.0	10.5	0.0	0.0
101	8.	2	146.0	137.0	15.2	0.0	0.0
102	8.	0	145.4	130.0	16.3	0.0	0.0
103	8.	0	147.4	72.0	9.0	0.0	0.0
104	8.	0	144.8	86.0	10.8	0.0	0.0

Table 3
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.04% Male Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
0	35.	0	104.3	539.0	15.4	1.5092	0.0431
1	35.	0	113.4	280.0	8.0	0.7837	0.0224
2	35.	0	111.3	276.0	7.9	0.7728	0.0221
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	126.0	0.0	0.0	0.0	0.0
5	35.	0	132.9	303.0	8.7	0.8484	0.0242
6	35.	0	134.8	280.0	8.0	0.7840	0.0224
7	35.	0	137.7	372.0	10.6	1.0416	0.0298
8	35.	0	137.7	297.0	8.5	0.8316	0.0238
9	35.	0	135.5	251.0	7.2	0.7028	0.0201
10	35.	0	135.5	354.0	10.1	0.9912	0.0283
11	35.	0	139.9	286.0	8.2	0.8008	0.0229
12	35.	0	138.8	305.0	8.7	0.8540	0.0244
13	35.	0	141.6	521.0	14.9	1.4535	0.0415
14	35.	0	140.8	272.0	7.8	0.7582	0.0217
15	35.	0	141.6	373.0	10.7	1.0415	0.0298
16	35.	0	140.1	281.0	8.0	0.7868	0.0225
17	35.	0	143.2	385.0	11.0	1.0787	0.0308
18	35.	0	144.5	510.0	14.6	1.4285	0.0408
19	35.	0	142.9	349.0	10.0	0.9741	0.0279
20	35.	0	146.1	349.0	10.0	0.9772	0.0279
21	35.	0	150.6	412.0	11.8	1.1536	0.0330
22	35.	0	155.1	416.0	11.9	1.2012	0.0343
23	35.	0	154.2	246.0	7.0	0.6884	0.0197
24	0.	0	0.0	0.0	0.0	0.0	0.0
25	35.	0	153.0	300.0	8.6	0.8380	0.0239
26	35.	0	152.2	356.0	10.2	0.9967	0.0285
27	34.	1	154.4	343.0	9.9	0.9593	0.0278
28	34.	0	153.8	307.0	9.0	0.8591	0.0253
29	34.	0	167.0	125.0	3.7	0.3503	0.0103
30	34.	0	153.2	375.0	11.0	1.0509	0.0309
31	34.	0	152.0	321.0	9.4	0.8964	0.0264
32	34.	0	153.7	388.0	11.4	1.0850	0.0319
33	34.	0	155.9	270.0	7.9	0.7558	0.0222
34	34.	0	154.3	356.0	10.5	0.9929	0.0292
35	34.	0	154.6	334.0	9.8	0.9330	0.0274
36	34.	0	155.0	318.0	9.4	0.8899	0.0262
37	33.	1	157.8	370.0	11.0	1.0343	0.0309
38	33.	0	155.5	367.0	11.1	1.0231	0.0310
39	33.	0	153.9	385.0	11.7	1.0778	0.0327
40	31.	2	151.8	385.0	12.0	1.0775	0.0337
41	31.	0	150.1	296.0	9.5	0.8315	0.0268
42	30.	1	148.8	420.0	13.8	1.1740	0.0385
43	30.	0	147.1	309.0	10.3	0.8639	0.0288
44	30.	0	146.0	321.0	10.7	0.8948	0.0298
45	30.	0	144.7	410.0	13.7	1.1507	0.0384
46	30.	0	144.0	389.0	13.0	1.0888	0.0363
47	30.	0	142.0	402.0	13.4	1.1270	0.0376
48	30.	0	143.1	480.0	16.0	1.3437	0.0448
49	30.	0	143.3	496.0	16.5	1.3871	0.0462
50	30.	0	141.8	404.0	13.5	1.1263	0.0375
51	30.	0	141.2	385.0	12.8	1.0780	0.0359
52	30.	0	141.2	379.0	12.6	1.0587	0.0353

Table 4
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.04% Male Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
53	28.	2	142.8	364.0	12.6	1.0192	0.0351
54	28.	0	142.5	373.0	13.3	1.0444	0.0373
55	28.	0	143.7	288.0	10.3	0.8064	0.0288
56	28.	0	140.5	267.0	9.5	0.7476	0.0267
57	27.	1	139.3	327.0	11.9	0.9168	0.0333
58	25.	2	143.4	357.0	13.7	0.9996	0.0384
59	25.	0	143.7	305.0	12.2	0.8546	0.0342
60	25.	0	144.5	406.0	16.2	1.1368	0.0455
61	25.	0	144.3	327.0	13.1	0.9156	0.0366
62	25.	0	144.8	327.0	13.1	0.9156	0.0366
63	25.	0	145.4	359.0	14.4	0.9352	0.0374
64	25.	0	144.8	357.0	14.3	0.9996	0.0400
65	24.	1	147.0	357.0	14.6	0.9996	0.0408
66	24.	0	146.8	383.0	16.0	1.0724	0.0447
67	23.	1	145.9	417.0	17.7	1.1676	0.0497
68	23.	0	134.3	354.0	15.4	0.9912	0.0431
69	23.	0	143.8	352.0	15.3	0.9912	0.0431
70	22.	1	141.8	286.0	12.7	0.8002	0.0356
71	22.	0	142.3	245.0	11.1	0.6860	0.0312
72	22.	0	138.6	229.0	10.4	0.6412	0.0291
73	21.	1	139.0	244.0	11.3	0.6832	0.0318
74	21.	0	137.6	281.0	13.4	0.7868	0.0375
75	20.	1	143.3	225.0	11.0	0.6300	0.0307
76	19.	1	142.4	182.0	9.3	0.4851	0.0249
77	18.	1	144.2	187.0	10.1	0.5080	0.0275
78	17.	1	141.5	186.0	10.6	0.5208	0.0298
79	17.	0	139.9	203.0	11.9	0.5684	0.0334
80	17.	0	138.6	190.0	11.2	0.5320	0.0313
81	16.	1	142.3	153.0	9.3	0.4284	0.0260
82	15.	1	142.3	109.0	7.0	0.3024	0.0195
83	14.	1	143.6	148.0	10.2	0.4144	0.0286
84	14.	0	143.5	121.0	8.6	0.3332	0.0238
85	14.	0	142.4	141.0	10.1	0.3948	0.0282
86	14.	0	139.6	170.0	12.1	0.4760	0.0340
87	14.	0	138.0	149.0	10.6	0.4172	0.0298
88	14.	0	133.4	170.0	12.1	0.4760	0.0340
89	12.	2	141.3	128.0	9.8	0.3584	0.0276
90	11.	1	137.7	142.0	12.3	0.3976	0.0346
91	10.	1	134.4	152.0	14.5	0.4256	0.0405
92	10.	0	134.3	100.0	10.0	0.2800	0.0280
93	10.	0	132.1	96.0	9.6	0.2688	0.0269
94	10.	0	126.0	139.0	13.9	0.3892	0.0389
95	8.	2	128.6	130.0	14.4	0.3640	0.0404
96	7.	1	124.9	117.0	15.6	0.3000	0.0400
97	6.	1	131.0	80.0	12.3	0.2240	0.0345
98	5.	1	136.0	122.0	22.2	0.3416	0.0621
99	5.	0	136.0	71.0	14.2	0.1988	0.0398
100	5.	0	135.4	78.0	15.6	0.2184	0.0437
101	5.	0	130.0	123.0	24.6	0.3444	0.0689
102	5.	0	130.2	94.0	18.8	0.2524	0.0505
103	5.	0	126.6	97.0	19.4	0.2716	0.0543
104	5.	0	122.0	66.0	13.2	0.1848	0.0370

Table 5
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.02% Male Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CC3/WEEK	AVG/ANIM
0	35.	0	96.7	689.0	19.7	0.9646	0.0276
1	35.	0	99.8	259.0	7.4	0.3786	0.0108
2	35.	0	107.5	292.0	8.3	0.4088	0.0117
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	119.0	0.0	0.0	0.0	0.0
5	35.	0	124.5	328.0	9.4	0.4592	0.0131
6	35.	0	122.2	308.0	8.8	0.4312	0.0123
7	35.	0	125.3	352.0	10.1	0.8204	0.0234
8	35.	0	126.3	289.0	8.3	0.4046	0.0116
9	35.	0	123.2	272.0	7.8	0.3408	0.0097
10	35.	0	122.4	360.0	10.3	0.5040	0.0144
11	35.	0	124.7	355.0	10.1	0.4970	0.0142
12	35.	0	126.5	323.0	9.2	0.4522	0.0129
13	34.	1	128.5	432.0	12.5	0.8780	0.0254
14	34.	0	129.0	325.0	9.6	0.4541	0.0134
15	34.	0	128.9	516.0	15.2	0.7223	0.0212
16	34.	0	126.4	396.0	11.6	0.5456	0.0160
17	34.	0	130.8	485.0	14.3	0.6780	0.0199
18	34.	0	133.7	555.0	16.3	0.7773	0.0229
19	34.	0	132.4	407.0	12.0	0.5701	0.0168
20	34.	0	134.3	400.0	11.8	0.5600	0.0165
21	34.	0	144.8	360.0	10.6	0.5040	0.0148
22	34.	0	142.7	450.0	13.2	0.6300	0.0185
23	34.	0	143.7	368.0	10.8	0.5143	0.0151
24	34.	0	142.6	215.0	6.3	0.3014	0.0089
25	34.	0	142.6	209.0	6.1	0.2914	0.0086
26	34.	0	142.5	291.0	8.6	0.4088	0.0120
27	34.	0	143.3	279.0	8.2	0.3901	0.0115
28	34.	0	142.0	237.0	7.0	0.3306	0.0097
29	34.	0	147.3	254.0	7.5	0.3545	0.0104
30	34.	0	144.0	329.0	9.7	0.4593	0.0135
31	34.	0	145.2	342.0	10.1	0.4785	0.0141
32	34.	0	148.1	329.0	9.7	0.4592	0.0135
33	34.	0	149.4	267.0	7.9	0.3746	0.0110
34	34.	0	147.4	278.0	8.2	0.3893	0.0114
35	34.	0	156.4	218.0	6.4	0.3034	0.0089
36	34.	0	148.1	306.0	9.0	0.4257	0.0125
37	34.	0	147.7	393.0	11.6	0.5502	0.0162
38	33.	1	147.8	399.0	11.9	0.5591	0.0167
39	33.	0	146.8	373.0	11.3	0.5218	0.0158
40	33.	0	144.5	404.0	12.2	0.5651	0.0171
41	33.	0	143.5	453.0	13.7	0.6352	0.0192
42	33.	0	142.0	418.0	12.7	0.5835	0.0177
43	33.	0	141.3	385.0	11.7	0.5369	0.0163
44	33.	0	138.9	376.0	11.4	0.5256	0.0159
45	33.	0	137.6	468.0	14.2	0.6536	0.0198
46	32.	1	136.0	427.0	13.1	0.5201	0.0160
47	32.	0	135.7	386.0	12.1	0.5397	0.0169
48	32.	0	137.8	612.0	19.1	0.8555	0.0267
49	32.	0	138.7	417.0	13.0	0.5835	0.0182
50	32.	0	137.3	364.0	11.4	0.5075	0.0159
51	32.	0	136.2	384.0	12.0	0.5392	0.0168
52	32.	0	136.8	411.0	12.8	0.5754	0.0180

Table 6
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.2% Male Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
53	32.	0	138.8	359.0	11.2	0.5023	0.0157
54	32.	0	139.8	356.0	11.1	0.4980	0.0156
55	32.	0	139.6	369.0	11.5	0.5166	0.0161
56	32.	0	137.9	293.0	9.2	0.4100	0.0128
57	31.	1	139.4	382.0	12.1	0.5348	0.0170
58	31.	0	139.6	350.0	11.3	0.4900	0.0158
59	31.	0	139.5	331.0	10.7	0.4634	0.0149
60	31.	0	140.0	361.0	11.6	0.5026	0.0162
61	31.	0	140.1	390.0	12.6	0.5460	0.0176
62	31.	0	140.9	319.0	10.3	0.4480	0.0145
63	31.	0	141.0	365.0	11.8	0.5110	0.0165
64	31.	0	140.5	365.0	11.8	0.5096	0.0164
65	31.	0	140.6	430.0	13.9	0.6020	0.0194
66	31.	0	141.5	429.0	13.8	0.6006	0.0194
67	31.	0	141.6	429.0	13.8	0.6006	0.0194
68	31.	0	126.4	365.0	11.8	0.5096	0.0164
69	29.	2	136.7	537.0	17.9	0.7518	0.0251
70	29.	0	134.6	292.0	10.1	0.4088	0.0141
71	27.	2	136.9	291.0	10.4	0.3794	0.0135
72	25.	2	136.0	268.0	10.3	0.3752	0.0144
73	24.	1	136.8	273.0	11.1	0.3822	0.0156
74	24.	0	135.6	265.0	11.0	0.3710	0.0155
75	24.	0	138.8	241.0	10.0	0.3374	0.0141
76	24.	0	137.8	188.0	7.8	0.2632	0.0110
77	23.	1	137.1	175.0	7.4	0.2450	0.0104
78	23.	0	133.3	193.0	8.4	0.2702	0.0117
79	23.	0	129.2	228.0	9.9	0.3192	0.0139
80	23.	0	131.1	209.0	9.1	0.2926	0.0127
81	23.	0	129.5	175.0	7.6	0.2450	0.0107
82	23.	0	128.5	143.0	6.2	0.2002	0.0087
83	23.	0	127.6	177.0	7.7	0.2478	0.0108
84	21.	2	130.3	157.0	7.1	0.2198	0.0100
85	21.	0	129.1	153.0	7.3	0.2142	0.0102
86	21.	0	131.1	213.0	10.1	0.2982	0.0142
87	20.	1	131.6	180.0	8.8	0.2520	0.0123
88	20.	0	129.2	231.0	11.5	0.3234	0.0162
89	19.	1	132.9	216.0	11.1	0.3024	0.0155
90	19.	0	131.2	235.0	12.4	0.3290	0.0173
91	18.	1	127.2	231.0	12.5	0.3234	0.0175
92	16.	2	129.4	143.0	8.4	0.2002	0.0118
93	16.	0	131.2	155.0	9.7	0.2170	0.0136
94	16.	0	124.4	138.0	8.6	0.1932	0.0121
95	16.	0	129.4	183.0	11.4	0.2562	0.0160
96	16.	0	128.9	123.0	7.7	0.1638	0.0102
97	15.	1	131.5	169.0	10.9	0.2366	0.0153
98	14.	1	131.4	175.0	12.1	0.2450	0.0169
99	14.	0	133.6	158.0	11.3	0.2212	0.0158
100	14.	0	129.3	123.0	8.8	0.1722	0.0123
101	14.	0	130.1	191.0	13.6	0.2674	0.0191
102	13.	1	127.8	201.0	14.9	0.2814	0.0208
103	12.	1	124.8	151.0	12.1	0.2114	0.0169
104	11.	1	124.0	111.0	9.7	0.1554	0.0135

Table 7
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.01% Male Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CDB/WEEK	AVG/ANIM
0	35.	0	104.1	586.0	16.7	0.4144	0.0118
1	35.	0	103.3	341.0	9.7	0.2387	0.0068
2	35.	0	111.1	344.0	9.8	0.2408	0.0069
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	121.9	0.0	0.0	0.0	0.0
5	35.	0	129.8	298.0	8.5	0.2086	0.0060
6	35.	0	132.3	287.0	8.2	0.2009	0.0057
7	35.	0	135.2	304.0	8.7	0.2408	0.0069
8	35.	0	134.1	313.0	8.9	0.2191	0.0063
9	35.	0	132.5	250.0	7.1	0.1820	0.0052
10	35.	0	135.8	364.0	10.4	0.2548	0.0073
11	35.	0	136.7	318.0	9.1	0.2226	0.0064
12	35.	0	138.0	276.0	7.9	0.1932	0.0055
13	35.	0	138.6	509.0	14.5	0.3563	0.0102
14	35.	0	140.5	319.0	9.1	0.2223	0.0064
15	35.	0	140.4	456.0	13.0	0.3194	0.0091
16	35.	0	137.1	292.0	8.3	0.1792	0.0051
17	35.	0	142.5	0.0	0.0	0.0	0.0
18	35.	0	144.3	410.0	11.7	0.2871	0.0082
19	35.	0	143.1	355.0	10.1	0.2477	0.0071
20	35.	0	144.9	406.0	11.6	0.2842	0.0081
21	35.	0	147.4	332.0	9.5	0.2324	0.0066
22	35.	0	152.6	449.0	12.8	0.3143	0.0090
23	35.	0	153.5	309.0	8.8	0.2169	0.0062
24	35.	0	151.7	296.0	8.5	0.1760	0.0050
25	35.	0	151.3	243.0	6.9	0.1700	0.0049
26	35.	0	149.6	366.0	10.5	0.2559	0.0073
27	35.	0	149.3	363.0	10.4	0.2136	0.0061
28	35.	0	146.9	259.0	7.4	0.1823	0.0052
29	35.	0	150.6	128.0	3.7	0.0995	0.0028
30	34.	1	148.6	533.0	15.4	0.3731	0.0108
31	34.	0	148.9	253.0	7.4	0.1768	0.0052
32	34.	0	142.8	284.0	8.4	0.1984	0.0058
33	34.	0	147.4	313.0	9.2	0.2179	0.0064
34	34.	0	147.7	260.0	7.6	0.1814	0.0053
35	34.	0	146.4	464.0	13.6	0.3243	0.0095
36	34.	0	147.2	385.0	11.3	0.2696	0.0079
37	34.	0	146.4	334.0	9.8	0.2325	0.0068
38	34.	0	145.4	414.0	12.2	0.2901	0.0085
39	34.	0	144.5	489.0	14.4	0.3415	0.0100
40	33.	1	144.8	415.0	12.4	0.2907	0.0087
41	33.	0	143.3	400.0	12.1	0.2800	0.0085
42	33.	0	141.9	437.0	13.2	0.3065	0.0093
43	32.	1	142.1	376.0	11.6	0.2103	0.0065
44	32.	0	138.0	398.0	12.4	0.2793	0.0087
45	32.	0	136.6	397.0	12.4	0.2785	0.0087
46	32.	0	135.8	370.0	11.6	0.2577	0.0081
47	31.	1	141.7	370.0	11.7	0.2589	0.0082
48	30.	1	141.5	514.0	16.9	0.3586	0.0118
49	30.	0	141.0	358.0	11.3	0.2360	0.0079
50	30.	0	140.4	377.0	12.6	0.2643	0.0088
51	30.	0	139.7	312.0	10.4	0.2114	0.0070
52	29.	1	141.2	387.0	13.1	0.2709	0.0092

Table 8
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.01% Male Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CDB/WEEK	AVG/ANIM
53	29.	0	141.8	363.0	12.5	0.2542	0.0066
54	29.	0	142.9	356.0	12.3	0.2492	0.0086
55	29.	0	142.6	328.0	11.3	0.2338	0.0081
56	29.	0	141.0	237.0	8.2	0.1659	0.0057
57	29.	0	140.3	349.0	12.0	0.2443	0.0084
58	29.	0	139.7	318.0	11.0	0.2225	0.0077
59	29.	0	139.3	337.0	11.6	0.2359	0.0081
60	28.	1	140.6	359.0	12.6	0.2513	0.0088
61	28.	0	140.5	396.0	14.1	0.2772	0.0099
62	28.	0	141.2	377.0	13.5	0.2639	0.0094
63	28.	0	140.7	354.0	12.6	0.2478	0.0088
64	28.	0	141.1	352.0	12.6	0.2464	0.0088
65	28.	0	141.4	445.0	15.9	0.3115	0.0111
66	27.	1	143.5	472.0	17.2	0.3304	0.0120
67	27.	0	142.6	470.0	17.4	0.3290	0.0122
68	26.	1	131.0	352.0	13.3	0.2464	0.0095
69	26.	0	140.3	386.0	14.8	0.2702	0.0104
70	25.	1	138.8	286.0	11.4	0.2002	0.0079
71	24.	1	138.6	283.0	11.6	0.2181	0.0089
72	24.	0	136.3	275.0	11.5	0.1925	0.0080
73	24.	0	137.6	319.0	13.3	0.2212	0.0092
74	22.	2	139.8	271.0	11.3	0.1897	0.0082
75	22.	0	143.0	240.0	10.9	0.1729	0.0079
76	22.	0	141.6	169.0	7.7	0.1183	0.0054
77	22.	0	139.9	169.0	7.7	0.1183	0.0054
78	22.	0	137.9	178.0	8.1	0.1246	0.0057
79	20.	2	139.0	171.0	8.1	0.1197	0.0057
80	20.	0	140.9	223.0	11.1	0.1561	0.0071
81	20.	0	137.3	153.0	7.6	0.1071	0.0054
82	18.	2	135.5	120.0	6.3	0.0840	0.0044
83	18.	2	139.8	150.0	8.8	0.1050	0.0062
84	14.	2	139.5	135.0	9.0	0.0945	0.0063
85	14.	0	136.5	181.0	12.9	0.1267	0.0090
86	13.	1	137.3	162.0	12.0	0.1134	0.0084
87	13.	0	137.5	135.0	10.4	0.0945	0.0073
88	13.	0	136.2	190.0	14.6	0.1330	0.0102
89	12.	1	137.6	161.0	12.9	0.1127	0.0090
90	12.	0	136.6	174.0	14.5	0.1218	0.0101
91	12.	0	135.7	155.0	12.9	0.1085	0.0090
92	12.	0	135.4	123.0	10.3	0.0861	0.0072
93	12.	0	134.9	134.0	11.2	0.0938	0.0078
94	12.	1	137.5	121.0	10.5	0.0847	0.0074
95	11.	0	140.1	132.0	12.0	0.0924	0.0084
96	10.	1	144.5	119.0	11.9	0.0833	0.0079
97	9.	1	147.6	112.0	11.8	0.0784	0.0083
98	9.	0	145.0	161.0	17.9	0.1127	0.0125
99	9.	0	148.1	125.0	13.9	0.0875	0.0097
100	9.	0	147.8	137.0	15.2	0.0959	0.0107
101	9.	0	143.1	179.0	19.9	0.1253	0.0139
102	9.	0	142.8	152.0	16.9	0.1064	0.0118
103	9.	0	144.6	113.0	12.6	0.0791	0.0088
104	9.	0	143.3	119.0	13.2	0.0833	0.0093

Table 9
Mean Average Weight, Feed and Compound Consumption
and Survival in Female Control Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
0	35.	0	103.3	380.0	10.9	0.0	0.0
1	35.	0	102.2	299.0	8.5	0.0	0.0
2	35.	0	116.8	276.0	7.9	0.0	0.0
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	138.7	0.0	0.0	0.0	0.0
5	35.	0	142.5	273.0	7.8	0.0	0.0
6	35.	0	140.4	271.0	7.7	0.0	0.0
7	35.	0	146.1	382.0	10.9	0.0	0.0
8	35.	0	148.2	409.0	11.7	0.0	0.0
9	35.	0	138.8	213.0	6.1	0.0	0.0
10	35.	0	145.3	375.0	10.7	0.0	0.0
11	35.	0	145.9	325.0	9.3	0.0	0.0
12	35.	0	145.3	282.0	8.1	0.0	0.0
13	35.	0	145.5	0.0	0.0	0.0	0.0
14	35.	0	144.3	250.0	7.1	0.0	0.0
15	35.	0	149.9	442.0	12.6	0.0	0.0
16	35.	0	145.4	539.0	15.4	0.0	0.0
17	35.	0	152.6	373.0	10.7	0.0	0.0
18	35.	0	154.5	426.0	12.2	0.0	0.0
19	35.	0	153.8	262.0	7.5	0.0	0.0
20	32.	3	163.7	318.0	9.5	0.0	0.0
21	32.	0	162.6	392.0	12.3	0.0	0.0
22	32.	0	166.0	408.0	12.8	0.0	0.0
23	32.	0	170.5	422.0	13.2	0.0	0.0
24	0.	0	0.0	0.0	0.0	0.0	0.0
25	32.	0	165.2	396.0	12.4	0.0	0.0
26	32.	0	156.3	397.0	12.4	0.0	0.0
27	32.	0	159.7	378.0	11.8	0.0	0.0
28	32.	0	160.7	313.0	9.8	0.0	0.0
29	32.	0	175.8	194.0	6.1	0.0	0.0
30	30.	2	169.1	430.0	13.9	0.0	0.0
31	30.	0	162.5	342.0	11.4	0.0	0.0
32	30.	0	164.4	364.0	12.1	0.0	0.0
33	29.	1	170.2	327.0	11.1	0.0	0.0
34	28.	1	169.5	254.0	8.9	0.0	0.0
35	27.	1	170.2	414.0	15.1	0.0	0.0
36	27.	0	170.8	344.0	12.7	0.0	0.0
37	27.	0	170.1	327.0	12.1	0.0	0.0
38	27.	0	167.0	322.0	11.9	0.0	0.0
39	27.	0	165.7	293.0	10.9	0.0	0.0
40	27.	0	161.9	378.0	14.0	0.0	0.0
41	26.	1	160.0	363.0	13.7	0.0	0.0
42	26.	0	157.7	406.0	15.6	0.0	0.0
43	25.	1	160.0	288.0	11.3	0.0	0.0
44	25.	0	156.2	322.0	12.9	0.0	0.0
45	25.	0	156.1	315.0	12.6	0.0	0.0
46	25.	0	154.8	325.0	13.0	0.0	0.0
47	25.	0	153.4	309.0	12.4	0.0	0.0
48	24.	1	152.4	394.0	16.1	0.0	0.0
49	24.	0	148.0	325.0	13.5	0.0	0.0
50	22.	2	148.4	284.0	12.3	0.0	0.0
51	22.	0	145.9	364.0	16.5	0.0	0.0
52	22.	0	146.8	373.0	17.0	0.0	0.0

Table 10
Mean Average Weight, Feed and Compound Consumption
and Survival in Female Control Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
53	21.	1	144.6	270.0	12.6	0.0	0.0
54	20.	1	143.3	233.0	11.4	0.0	0.0
55	20.	0	142.4	228.0	11.4	0.0	0.0
56	19.	1	144.7	198.0	10.2	0.0	0.0
57	18.	1	142.8	255.0	13.8	0.0	0.0
58	18.	0	141.7	210.0	11.7	0.0	0.0
59	15.	3	150.5	191.0	11.6	0.0	0.0
60	15.	0	150.6	215.0	14.3	0.0	0.0
61	15.	0	151.9	209.0	13.9	0.0	0.0
62	14.	1	154.4	210.0	14.5	0.0	0.0
63	13.	1	156.4	181.0	13.4	0.0	0.0
64	13.	0	150.2	178.0	13.7	0.0	0.0
65	12.	1	142.3	158.0	12.6	0.0	0.0
66	11.	1	143.4	142.0	12.3	0.0	0.0
67	10.	1	141.9	147.0	14.0	0.0	0.0
68	8.	2	136.8	151.0	16.8	0.0	0.0
69	8.	0	144.9	115.0	14.4	0.0	0.0
70	7.	1	144.0	91.0	12.1	0.0	0.0
71	7.	0	141.7	70.0	10.0	0.0	0.0
72	7.	0	135.6	60.0	8.6	0.0	0.0
73	7.	0	128.0	76.0	10.9	0.0	0.0
74	6.	1	132.7	67.0	10.3	0.0	0.0
75	6.	0	130.7	56.0	9.3	0.0	0.0
76	5.	1	137.2	30.0	5.5	0.0	0.0
77	4.	1	137.0	33.0	7.3	0.0	0.0
78	4.	0	137.8	34.0	8.5	0.0	0.0
79	4.	0	136.8	41.0	10.3	0.0	0.0
80	4.	0	137.5	44.0	11.0	0.0	0.0
81	4.	0	137.8	34.0	8.5	0.0	0.0
82	4.	0	135.5	32.0	8.0	0.0	0.0
83	4.	0	134.3	37.0	9.3	0.0	0.0
84	4.	0	136.0	26.0	6.5	0.0	0.0
85	2.	2	142.0	21.0	7.0	0.0	0.0
86	1.	1	140.0	10.0	6.7	0.0	0.0
87	1.	0	128.0	12.0	12.0	0.0	0.0
88	0.	0	0.0	0.0	0.0	0.0	0.0
89	0.	0	0.0	0.0	0.0	0.0	0.0
90	0.	0	0.0	0.0	0.0	0.0	0.0
91	0.	0	0.0	0.0	0.0	0.0	0.0
92	0.	0	0.0	0.0	0.0	0.0	0.0
93	0.	0	0.0	0.0	0.0	0.0	0.0
94	0.	0	0.0	0.0	0.0	0.0	0.0
95	0.	0	0.0	0.0	0.0	0.0	0.0
96	0.	0	0.0	0.0	0.0	0.0	0.0
97	0.	0	0.0	0.0	0.0	0.0	0.0
98	0.	0	0.0	0.0	0.0	0.0	0.0
99	0.	0	0.0	0.0	0.0	0.0	0.0
100	0.	0	0.0	0.0	0.0	0.0	0.0
101	0.	0	0.0	0.0	0.0	0.0	0.0
102	0.	0	0.0	0.0	0.0	0.0	0.0
103	0.	0	0.0	0.0	0.0	0.0	0.0
104	0.	0	0.0	0.0	0.0	0.0	0.0

Table 11
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.04% Female Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FUOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
0	35.	0	105.1	623.0	17.8	1.7444	0.0498
1	35.	0	113.9	305.0	8.7	0.8540	0.0244
2	35.	0	120.6	278.0	7.9	0.7784	0.0222
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	137.5	0.0	0.0	0.0	0.0
5	35.	0	144.9	326.0	9.3	0.9128	0.0261
6	35.	0	141.7	321.0	9.2	0.8988	0.0257
7	35.	0	146.5	481.0	13.7	1.3468	0.0385
8	35.	0	150.2	475.0	13.6	1.3300	0.0380
9	35.	0	142.3	366.0	10.5	1.0528	0.0301
10	35.	0	147.0	429.0	12.3	1.2012	0.0343
11	35.	0	147.2	408.0	11.7	1.1424	0.0326
12	35.	0	146.7	367.0	10.5	1.0276	0.0294
13	35.	0	153.4	547.0	15.6	1.5303	0.0437
14	35.	0	152.3	337.0	9.6	0.9409	0.0269
15	35.	0	153.5	449.0	12.8	1.2573	0.0359
16	35.	0	149.4	324.0	9.3	0.9072	0.0259
17	35.	0	155.4	534.0	15.3	1.4954	0.0427
18	35.	0	159.8	456.0	13.0	1.2810	0.0366
19	35.	0	160.2	295.0	8.4	1.2360	0.0353
20	35.	0	160.4	307.0	8.8	0.8596	0.0246
21	35.	0	165.1	410.0	11.7	1.1480	0.0328
22	35.	0	171.6	492.0	14.1	1.3776	0.0394
23	35.	0	173.4	452.0	12.9	1.2664	0.0362
24	0.	0	0.0	0.0	0.0	0.0	0.0
25	35.	0	168.4	419.0	12.0	1.1726	0.0335
26	35.	0	162.2	373.0	10.7	1.0444	0.0298
27	35.	0	166.9	383.0	10.9	1.0690	0.0305
28	35.	0	166.5	354.0	10.1	0.9921	0.0283
29	34.	1	184.1	203.0	5.9	0.5673	0.0164
30	34.	0	170.6	478.0	14.1	1.3395	0.0394
31	34.	0	165.9	362.0	10.6	1.0125	0.0298
32	34.	0	165.3	340.0	10.0	0.9515	0.0280
33	33.	1	171.9	296.0	8.8	0.8288	0.0247
34	33.	0	170.9	394.0	11.9	1.1023	0.0334
35	32.	1	168.9	407.0	12.5	1.1381	0.0350
36	32.	0	169.7	353.0	11.0	0.9858	0.0308
37	32.	0	168.5	372.0	11.6	1.0416	0.0325
38	32.	0	167.3	424.0	13.3	1.1892	0.0372
39	32.	0	167.1	481.0	15.0	1.3468	0.0421
40	32.	0	162.6	435.0	13.6	1.2202	0.0381
41	32.	0	162.0	406.0	12.7	1.1359	0.0355
42	31.	1	157.9	472.0	15.0	1.3188	0.0419
43	30.	1	155.9	351.0	11.5	0.9806	0.0322
44	30.	0	152.3	411.0	13.7	1.1469	0.0382
45	30.	0	150.1	403.0	13.4	1.1246	0.0375
46	30.	0	149.4	416.0	13.9	1.1643	0.0388
47	30.	0	148.4	435.0	14.5	1.2184	0.0406
48	29.	1	146.0	473.0	16.0	1.3246	0.0449
49	28.	1	144.4	461.0	16.2	1.2895	0.0452
50	28.	0	145.3	406.0	14.5	1.1358	0.0406
51	28.	0	144.9	432.0	15.4	1.2096	0.0432
52	28.	0	145.3	451.0	16.1	1.2628	0.0451

Table 12
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.04% Female Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
53	28.	0	145.6	397.0	14.2	1.1122	0.0397
54	27.	1	146.9	336.0	12.2	0.9408	0.0342
55	27.	0	145.7	272.0	10.1	0.7616	0.0282
56	27.	0	144.0	259.0	9.6	0.7252	0.0269
57	27.	0	142.9	358.0	13.3	1.0024	0.0371
58	27.	0	141.6	354.0	13.1	0.9912	0.0367
59	26.	1	138.2	293.0	11.1	0.8211	0.0310
60	24.	2	138.5	357.0	14.3	0.9996	0.0400
61	23.	1	140.1	359.0	15.3	1.0052	0.0428
62	22.	1	140.9	297.0	13.2	0.8316	0.0370
63	21.	1	140.3	332.0	15.4	0.9296	0.0432
64	20.	1	142.4	284.0	13.0	0.7952	0.0388
65	19.	1	144.7	332.0	17.0	0.9296	0.0477
66	19.	0	145.1	357.0	18.8	0.9996	0.0526
67	19.	0	141.7	324.0	17.1	0.9072	0.0477
68	19.	0	131.4	267.0	14.1	0.7476	0.0393
69	18.	1	137.4	242.0	13.1	0.6776	0.0366
70	16.	2	131.9	249.0	14.6	0.6972	0.0410
71	14.	2	134.8	236.0	15.7	0.6608	0.0441
72	14.	0	130.6	213.0	15.2	0.5964	0.0426
73	12.	2	130.1	211.0	16.2	0.5908	0.0454
74	11.	1	129.5	210.0	18.3	0.5880	0.0511
75	8.	3	123.8	145.0	15.3	0.4060	0.0427
76	7.	1	116.7	90.0	12.0	0.2520	0.0336
77	5.	2	113.8	68.0	11.3	0.1904	0.0317
78	5.	0	109.4	54.0	10.8	0.1512	0.0302
79	3.	2	107.0	44.0	11.0	0.1232	0.0308
80	1.	2	159.0	12.0	6.0	0.0336	0.0168
81	1.	0	159.0	19.0	19.0	0.0532	0.0532
82	1.	0	155.0	13.0	13.0	0.0364	0.0364
83	1.	0	152.0	12.0	12.0	0.0336	0.0336
84	1.	0	143.0	16.0	16.0	0.0448	0.0448
85	1.	0	141.0	21.0	21.0	0.0588	0.0588
86	1.	0	131.0	23.0	23.0	0.0644	0.0644
87	1.	0	124.0	18.0	18.0	0.0504	0.0504
88	1.	0	118.0	22.0	22.0	0.0616	0.0616
89	1.	0	122.0	14.0	14.0	0.0392	0.0392
90	1.	0	115.0	10.0	10.0	0.0280	0.0280
91	1.	0	118.0	11.0	11.0	0.0308	0.0308
92	0.	0	0.0	0.0	0.0	0.0	0.0
93	0.	0	0.0	0.0	0.0	0.0	0.0
94	0.	0	0.0	0.0	0.0	0.0	0.0
95	0.	0	0.0	0.0	0.0	0.0	0.0
96	0.	0	0.0	0.0	0.0	0.0	0.0
97	0.	0	0.0	0.0	0.0	0.0	0.0
98	0.	0	0.0	0.0	0.0	0.0	0.0
99	0.	0	0.0	0.0	0.0	0.0	0.0
100	0.	0	0.0	0.0	0.0	0.0	0.0
101	0.	0	0.0	0.0	0.0	0.0	0.0
102	0.	0	0.0	0.0	0.0	0.0	0.0
103	0.	0	0.0	0.0	0.0	0.0	0.0
104	0.	0	0.0	0.0	0.0	0.0	0.0

Table 13
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.02% Female Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
0	35.	0	106.0	391.0	11.2	0.5474	0.0156
1	35.	0	103.6	318.0	9.1	0.4984	0.0142
2	35.	0	117.7	300.0	8.6	0.4200	0.0120
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	130.4	0.0	0.0	0.0	0.0
5	35.	0	139.0	356.0	10.2	0.4984	0.0142
6	35.	0	138.3	358.0	10.2	0.5012	0.0143
7	35.	0	143.4	418.0	11.9	1.1704	0.0334
8	35.	0	141.6	386.0	11.0	0.5404	0.0154
9	35.	0	136.5	309.0	8.8	0.4446	0.0127
10	35.	0	140.1	467.0	13.3	0.6538	0.0187
11	35.	0	139.8	415.0	11.9	0.5810	0.0166
12	35.	0	136.5	324.0	9.3	0.4522	0.0129
13	35.	0	145.0	573.0	16.4	0.8024	0.0229
14	35.	0	146.9	512.0	14.6	0.7168	0.0205
15	35.	0	145.4	590.0	16.9	0.8240	0.0235
16	35.	0	143.2	371.0	10.6	0.5180	0.0148
17	35.	0	147.9	509.0	14.5	0.7116	0.0203
18	34.	1	150.5	401.0	11.6	0.5598	0.0162
19	34.	0	148.5	402.0	11.8	0.5617	0.0165
20	33.	1	150.2	459.0	13.7	0.6426	0.0192
21	32.	1	155.5	428.0	13.2	0.5992	0.0184
22	29.	3	156.5	517.0	17.0	0.7238	0.0237
23	27.	2	169.5	426.0	15.2	0.5967	0.0213
24	27.	0	167.5	309.0	11.4	0.4312	0.0160
25	27.	0	172.1	327.0	12.1	0.4600	0.0170
26	27.	0	166.3	332.0	12.3	0.4612	0.0171
27	27.	0	173.1	353.0	13.1	0.4952	0.0183
28	27.	0	172.9	263.0	9.7	0.3650	0.0135
29	27.	0	186.9	201.0	7.4	0.2805	0.0104
30	27.	0	174.4	366.0	13.6	0.5907	0.0219
31	27.	0	172.7	332.0	12.3	0.4646	0.0172
32	27.	0	172.4	295.0	10.9	0.4127	0.0153
33	27.	0	173.6	228.0	8.4	0.3182	0.0118
34	27.	0	171.3	285.0	10.6	0.3991	0.0148
35	26.	1	170.9	234.0	8.8	0.3280	0.0124
36	26.	0	171.0	293.0	11.3	0.4080	0.0157
37	26.	0	168.9	308.0	11.8	0.4287	0.0165
38	26.	0	168.1	339.0	13.0	0.4742	0.0182
39	26.	0	166.9	322.0	12.4	0.4497	0.0173
40	26.	0	161.2	340.0	13.1	0.4757	0.0183
41	26.	0	158.6	420.0	16.2	0.4892	0.0188
42	26.	0	154.4	398.0	15.3	0.5564	0.0214
43	25.	1	155.0	479.0	18.8	0.6712	0.0263
44	25.	0	148.8	276.0	11.0	0.3871	0.0155
45	25.	0	146.9	343.0	13.7	0.4796	0.0192
46	25.	0	146.6	360.0	14.4	0.5038	0.0202
47	24.	1	143.9	403.0	16.4	0.5642	0.0230
48	24.	0	141.1	405.0	16.9	0.5666	0.0236
49	24.	0	141.0	269.0	11.2	0.3749	0.0156
50	24.	0	141.8	315.0	13.1	0.4397	0.0183
51	24.	0	139.3	293.0	12.2	0.4102	0.0171
52	24.	0	140.7	333.0	13.9	0.4662	0.0194

Table 14
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.02% Female Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
53	24.	0	141.0	310.0	12.9	0.4340	0.0181
54	23.	1	140.3	357.0	15.2	0.4998	0.0213
55	23.	0	140.0	356.0	15.5	0.4984	0.0217
56	23.	0	138.7	426.0	18.5	0.5964	0.0259
57	22.	1	137.9	393.0	17.5	0.5502	0.0245
58	22.	0	135.5	310.0	14.1	0.4340	0.0197
59	22.	0	133.2	273.0	12.4	0.3822	0.0174
60	22.	0	130.3	302.0	13.7	0.4228	0.0192
61	18.	4	134.6	320.0	16.0	0.4480	0.0224
62	16.	2	137.3	290.0	17.1	0.4060	0.0239
63	15.	1	138.8	299.0	19.3	0.4186	0.0270
64	14.	1	136.9	273.0	18.8	0.3824	0.0264
65	14.	0	135.9	233.0	16.6	0.3262	0.0233
66	13.	1	135.5	212.0	15.7	0.2968	0.0220
67	11.	2	135.0	241.0	20.1	0.3374	0.0281
68	8.	3	136.8	143.0	15.1	0.2002	0.0211
69	6.	2	135.5	94.0	13.4	0.1316	0.0188
70	5.	1	147.6	86.0	15.6	0.1204	0.0219
71	5.	0	145.6	57.0	11.4	0.0798	0.0160
72	5.	0	138.8	102.0	20.4	0.1428	0.0286
73	5.	0	136.2	95.0	19.0	0.1330	0.0266
74	5.	0	134.4	102.0	20.4	0.1428	0.0286
75	5.	0	131.4	92.0	18.4	0.1288	0.0258
76	5.	0	129.2	44.0	8.8	0.0616	0.0123
77	4.	1	132.0	59.0	13.1	0.0826	0.0184
78	4.	0	131.3	58.0	14.5	0.0812	0.0203
79	4.	0	128.5	47.0	11.8	0.0658	0.0164
80	3.	1	127.0	55.0	15.7	0.0770	0.0220
81	2.	1	143.5	26.0	10.4	0.0364	0.0146
82	2.	0	136.5	33.0	16.5	0.0462	0.0231
83	2.	0	130.5	36.0	18.0	0.0504	0.0252
84	2.	0	124.0	39.0	19.5	0.0546	0.0273
85	2.	0	129.5	36.0	18.0	0.0504	0.0252
86	2.	0	132.5	27.0	13.5	0.0378	0.0189
87	1.	1	106.0	7.0	4.7	0.0	0.0
88	1.	0	114.0	29.0	29.0	0.0406	0.0406
89	1.	0	107.0	9.0	9.0	0.0126	0.0126
90	0.	0	0.0	0.0	0.0	0.0	0.0
91	0.	0	0.0	0.0	0.0	0.0	0.0
92	0.	0	0.0	0.0	0.0	0.0	0.0
93	0.	0	0.0	0.0	0.0	0.0	0.0
94	0.	0	0.0	0.0	0.0	0.0	0.0
95	0.	0	0.0	0.0	0.0	0.0	0.0
96	0.	0	0.0	0.0	0.0	0.0	0.0
97	0.	0	0.0	0.0	0.0	0.0	0.0
98	0.	0	0.0	0.0	0.0	0.0	0.0
99	0.	0	0.0	0.0	0.0	0.0	0.0
100	0.	0	0.0	0.0	0.0	0.0	0.0
101	0.	0	0.0	0.0	0.0	0.0	0.0
102	0.	0	0.0	0.0	0.0	0.0	0.0
103	0.	0	0.0	0.0	0.0	0.0	0.0
104	0.	0	0.0	0.0	0.0	0.0	0.0

Table 15
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.01% Female Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
0	35.	0	108.3	347.0	9.9	0.2429	0.0069
1	35.	0	107.9	352.0	10.1	0.2464	0.0070
2	35.	0	122.5	262.0	7.5	0.1834	0.0052
3	0.	0	0.0	0.0	0.0	0.0	0.0
4	35.	0	138.0	0.0	0.0	0.0	0.0
5	35.	0	145.8	327.0	9.3	0.2289	0.0065
6	35.	0	145.7	336.0	9.6	0.2352	0.0067
7	35.	0	152.5	436.0	12.5	0.4172	0.0119
8	35.	0	154.2	455.0	13.0	0.2744	0.0078
9	35.	0	146.2	371.0	10.6	0.2597	0.0074
10	35.	0	151.4	506.0	14.5	0.3604	0.0103
11	35.	0	147.7	374.0	10.7	0.2618	0.0075
12	35.	0	149.4	381.0	10.9	0.2667	0.0076
13	35.	0	155.2	620.0	17.7	0.4331	0.0124
14	35.	0	156.6	414.0	11.8	0.2895	0.0083
15	35.	0	158.9	583.0	16.7	0.3512	0.0100
16	35.	0	153.2	334.0	9.5	0.2338	0.0067
17	34.	1	163.4	0.0	0.0	0.0	0.0
18	34.	0	168.0	503.0	14.8	0.3533	0.0104
19	34.	0	168.6	407.0	12.0	0.2851	0.0084
20	34.	0	170.6	457.0	13.4	0.3199	0.0094
21	34.	0	175.2	411.0	12.1	0.2877	0.0085
22	34.	0	180.1	510.0	15.0	0.3570	0.0105
23	34.	0	178.6	465.0	13.7	0.3252	0.0096
24	34.	0	179.8	342.0	10.1	0.2391	0.0070
25	34.	0	180.3	396.0	11.6	0.2777	0.0082
26	33.	1	173.8	511.0	15.3	0.3571	0.0107
27	33.	0	177.7	374.0	11.3	0.2619	0.0079
28	33.	0	178.7	280.0	8.5	0.1958	0.0059
29	33.	0	186.8	271.0	8.2	0.1886	0.0057
30	33.	0	181.5	446.0	13.5	0.3122	0.0095
31	33.	0	179.3	338.0	10.2	0.2356	0.0071
32	32.	1	177.4	324.0	10.0	0.2273	0.0070
33	32.	0	182.4	369.0	11.5	0.2586	0.0081
34	32.	0	180.3	298.0	9.3	0.2082	0.0065
35	32.	0	178.1	444.0	13.9	0.3103	0.0097
36	32.	0	178.8	364.0	11.4	0.2552	0.0080
37	32.	0	178.9	353.0	11.0	0.2470	0.0077
38	31.	1	177.9	416.0	13.2	0.2908	0.0092
39	31.	0	177.5	430.0	13.9	0.3011	0.0097
40	31.	0	172.3	380.0	12.3	0.2660	0.0086
41	30.	1	171.4	370.0	12.1	0.2585	0.0085
42	30.	0	169.7	433.0	14.4	0.3021	0.0101
43	30.	0	167.9	390.0	13.0	0.2726	0.0091
44	30.	0	164.5	354.0	11.8	0.2469	0.0082
45	30.	0	162.0	404.0	13.5	0.2818	0.0094
46	29.	1	162.2	382.0	12.9	0.2670	0.0091
47	29.	0	160.5	415.0	14.3	0.2904	0.0100
48	28.	1	162.0	573.0	20.1	0.4007	0.0141
49	28.	0	159.7	462.0	16.5	0.3233	0.0115
50	28.	0	161.3	335.0	12.0	0.2341	0.0084
51	27.	1	162.5	363.0	13.2	0.2541	0.0092
52	27.	0	161.0	401.0	14.9	0.2807	0.0104

Table 16
Mean Average Weight, Feed and Compound Consumption
and Survival in 0.01% Female Group

WEEK	NO. ANIMALS	DEATHS	AVG WT	FOOD/24HR	AVG/ANIM	CD3/WEEK	AVG/ANIM
53	26.	1	164.2	359.0	13.5	0.2514	0.0095
54	26.	0	164.2	285.0	11.0	0.1994	0.0077
55	26.	0	164.3	309.0	11.9	0.5817	0.0224
56	25.	1	162.7	241.0	9.5	0.1687	0.0066
57	24.	1	161.8	301.0	12.3	0.2105	0.0086
58	23.	1	164.4	260.0	11.1	0.1820	0.0077
59	22.	1	166.3	254.0	11.3	0.1778	0.0079
60	22.	0	164.3	305.0	13.9	0.2135	0.0097
61	21.	1	168.3	292.0	13.6	0.2044	0.0095
62	21.	0	167.3	282.0	13.4	0.1575	0.0075
63	21.	0	162.8	269.0	12.8	0.1883	0.0090
64	21.	0	158.9	250.0	11.9	0.1750	0.0083
65	19.	2	162.6	299.0	14.9	0.2093	0.0105
66	19.	0	164.7	345.0	18.2	0.2415	0.0127
67	17.	2	166.9	280.0	15.6	0.1960	0.0109
68	17.	0	152.8	250.0	14.7	0.1750	0.0103
69	15.	2	151.7	257.0	16.1	0.1769	0.0111
70	12.	3	147.9	160.0	11.9	0.1090	0.0081
71	10.	2	152.2	123.0	11.2	0.0861	0.0078
72	10.	0	151.7	109.0	10.9	0.0763	0.0076
73	9.	1	149.6	137.0	14.4	0.0939	0.0099
74	7.	2	151.4	113.0	14.1	0.0791	0.0099
75	7.	0	149.4	95.0	13.6	0.0665	0.0095
76	7.	0	146.4	82.0	11.7	0.0574	0.0082
77	7.	0	141.0	62.0	8.9	0.0434	0.0062
78	7.	0	140.4	107.0	15.3	0.0749	0.0107
79	6.	1	142.7	83.0	12.8	0.0581	0.0089
80	6.	0	143.7	107.0	17.8	0.0749	0.0125
81	5.	1	146.2	67.0	12.2	0.0469	0.0085
82	4.	1	141.5	43.0	9.6	0.0301	0.0067
83	4.	0	146.8	51.0	12.8	0.0357	0.0089
84	4.	0	149.8	45.0	11.3	0.0315	0.0079
85	4.	0	151.5	68.0	17.0	0.0476	0.0119
86	3.	1	139.0	56.0	16.0	0.0392	0.0112
87	3.	0	135.0	37.0	12.3	0.0	0.0
88	3.	0	136.7	45.0	15.0	0.0315	0.0105
89	2.	1	123.0	12.0	4.8	0.0084	0.0034
90	0.	0	0.0	0.0	0.0	0.0	0.0
91	0.	0	0.0	0.0	0.0	0.0	0.0
92	0.	0	0.0	0.0	0.0	0.0	0.0
93	0.	0	0.0	0.0	0.0	0.0	0.0
94	0.	0	0.0	0.0	0.0	0.0	0.0
95	0.	0	0.0	0.0	0.0	0.0	0.0
96	0.	0	0.0	0.0	0.0	0.0	0.0
97	0.	0	0.0	0.0	0.0	0.0	0.0
98	0.	0	0.0	0.0	0.0	0.0	0.0
99	0.	0	0.0	0.0	0.0	0.0	0.0
100	0.	0	0.0	0.0	0.0	0.0	0.0
101	0.	0	0.0	0.0	0.0	0.0	0.0
102	0.	0	0.0	0.0	0.0	0.0	0.0
103	0.	0	0.0	0.0	0.0	0.0	0.0
104	0.	0	0.0	0.0	0.0	0.0	0.0

SUMMARY OF GROSS PATHOLOGY INCIDENCE TABLE 17

	0.00		0.04		0.02		0.01	
	M	F	M	F	M	F	M	F
LYMPH NODE	35	35	35	35	33	35	35	35
Inguinal enlarged	11	3	13	10	11	2	14	6
Axillary	0	0	0	0	0	0	1	1
Cervical enlarged	2	1	8	3	1	6	2	2
Mesenteric	0	0	0	0	0	0	2	0
Lumbar enlarged	0	0	0	0	1	0	0	0
Renal	0	0	1	0	0	0	0	0
LUNGS	35	35	35	35	33	35	35	35
Hemorrhage	16	0	8	12	11	7	17	11
Congestion	17	0	17	11	15	9	12	6
Emphysema	2	2	2	4	2	1	2	4
Pneumonia	0	0	0	1	0	0	0	0
Pale	0	0	0	0	1	0	1	0
Mass	0	0	0	0	0	1	0	0
Mottled	4	0	2	4	0	2	5	2
Exudate	1	0	0	0	0	0	0	0
URINARY BLADDER	35	35	35	35	33	34	35	35
Filled	20	2	15	0	13	1	23	6
Metaplasia	0	0	0	0	2	0	0	0
STOMACH	35	35	35	35	33	35	35	35
Ulcers	1	0	1	0	2	2	0	1
Mass	2	0	0	0	0	0	1	0
Discoloration	2	0	0	0	1	0	0	0
Bloat	0	0	0	0	0	0	1	0
Hemorrhage	1	0	0	0	0	0	0	0
INTESTINE	34	35	35	35	33	35	35	35
Bloated	8	2	1	0	0	1	7	3
Ulcers	0	0	0	0	1	0	0	0
Abscess	0	0	0	0	1	0	0	0
Hemorrhagic	1	1	0	0	0	2	3	0
Nodules	1	0	0	0	0	0	0	0
LIVER	35	35	35	35	33	35	35	35
Mottled	5	11	9	10	10	8	5	7
Lobular pattern	0	6	5	8	7	7	4	5
Enlarged	2	0	0	6	0	0	1	2
Pale	0	3	6	3	12	3	6	8
Congested	1	2	0	14	0	0	3	0
Cyst	7	8	3	12	6	13	6	11
Necrosis	0	0	0	0	0	0	3	1
Granular	1	2	1	2	1	1	3	0
Hemorrhagic	0	0	0	0	0	0	1	2
Abscess	1	0	0	0	0	0	0	0
Mass	0	0	0	0	0	0	1	0

SUMMARY OF GROSS PATHOLOGY INCIDENCE TABLE 18

	0.00		0.04		0.02		0.01	
	M	F	M	F	M	F	M	F
GALL BLADDER	35	35	35	35	33	35	35	35
Distended with fluid	0	0	0	0	1	0	0	0
KIDNEYS	35	35	35	35	33	35	35	35
Granular	14	18	16	19	17	8	20	14
Pale	18	5	14	16	17	3	11	8
Mottled	2	1	0	2	0	0	2	1
Pitted	0	0	0	0	3	0	0	0
Misshapen	0	1	0	0	0	0	0	0
Congestion	0	0	0	1	0	0	0	0
Large	2	0	1	3	0	0	0	0
Nodular	0	2	0	0	0	0	1	1
Dark	0	0	2	0	0	0	0	0
Infarct	1	0	1	1	0	1	0	0
Mass	0	1	0	0	0	0	1	0
SPLEEN	35	35	35	35	33	35	35	35
Enlarged	12	2	1	0	13	1	9	3
Congestion	0	0	0	0	0	0	2	1
SKIN	35	35	35	35	33	35	35	35
Edema	3	5	3	1	0	2	0	0
Mass	0	0	0	0	2	0	0	0
Dry	0	0	0	0	1	0	0	0
Hair loss	1	0	0	0	0	0	0	0
ABDOMINAL CAVITY	35	35	35	35	33	35	35	35
Fluid	4	1	2	3	0	2	0	2
Mass	0	0	0	0	0	0	0	1
SEMINAL VESICLES	35	0	35	0	33	0	35	0
Hemorrhage	1	0	0	0	0	0	0	0
OVARIES	0	35	0	35	0	35	0	35
Cyst	0	0	0	1	0	1	0	2
Enlarged	0	0	0	1	0	0	0	0
THORACIC CAVITY	35	35	35	35	33	35	35	35
Fluid	2	3	2	3	0	1	2	1
SALIVARY GLAND	31	35	32	34	33	35	35	32
Enlarged	0	1	0	0	0	0	0	0
UTERUS	0	35	0	35	0	35	0	35
Discoloration	0	1	0	0	0	0	0	0
ADRENAL	35	35	35	35	33	35	35	35
Enlarged	0	0	2	0	0	0	1	1
Pale	0	0	1	0	0	0	1	0

SUMMARY OF GROSS PATHOLOGY INCIDENCE TABLE 19

	0.00		0.04		0.02		0.01	
	M	F	M	F	M	F	M	F
TESTES	35	0	35	0	33	0	35	0
Atrophic	0	0	2	0	0	0	1	0
Abscess.	0	0	1	0	0	0	0	0
THYMUS	35	35	35	35	33	35	35	35
Enlarged	0	0	0	0	0	0	1	0
PITUITARY	35	35	35	35	33	35	35	35
Enlarged	0	0	0	0	0	0	1	0

NEOPLASM SUMMARY TABLE 20

Group:	0.00%		0.04%		0.02%		0.01%	
	M	F	M	F	M	F	M	F
LARYNX	11	20	18	27	25	32	28	25
Lymphoma	0	0	0	0	1	0	0	0
Papilloma	0	0	0	0	1	0	0	0
STOMACH	20	35	23	8	34	17	33	33
Papilloma	0	0	0	1	0	0	0	0
INTESTINES	26	32	23	13	34	19	33	33
Lymphoma - metastatic	0	0	0	0	0	0	1	0
LIVER	27	35	24	35	34	29	34	33
Lymphoma - metastatic	0	0	0	1	1	3	0	0
Hemangioma	0	0	0	0	0	2	2	0
Adenocarcinoma - metastatic	0	0	0	0	0	0	0	1
Angiosarcoma	0	0	0	0	0	0	1	0
SPLEEN	24	33	24	17	33	18	30	34
Lymphoma - metastatic	0	2	0	0	0	1	0	0
Hyperplastic nodule	0	0	1	0	0	0	0	0
LYMPH NODES	5	5	7	6	11	6	9	6
Lymphoma	0	1	1	1	0	0	0	1
Adenocarcinoma - metastatic	0	0	0	0	0	0	0	1
ADRENAL GLAND	23	34	17	13	17	12	18	28
Cortical adenoma	1	0	2	1	2	0	2	0
Cortical carcinoma	0	1	0	0	0	0	0	0
PANCREAS	6	27	11	11	7	11	8	8
Adenocarcinoma	0	0	0	0	0	0	0	1
THYROID GLAND	11	25	10	23	30	31	26	23
Lymphoma - metastatic	0	0	0	0	1	0	0	0
PARATHYROID GLAND	0	0	0	0	0	1	1	0
Adenoma	0	0	0	0	0	1	1	0
OVARY	0	29	0	4	0	23	0	29
Hemangioma	0	0	0	0	0	1	0	0
SKIN	0	0	1	0	0	0	0	0
Melanoma	0	0	1	0	0	0	0	0

SUMMARY OF HISTOPATHOLOGY INCIDENCE TABLE 21

Group:	0.00%		0.04%		0.02%		0.01%	
	M	F	M	F	M	F	M	F
LARYNX	11	20	18	27	25	32	28	25
Epithelial dysplasia	0	0	0	0	1	0	2	0
Desquamation	0	0	1	0	0	0	0	0
Degeneration	1	0	1	0	0	0	1	0
Inflammation	0	0	0	0	0	1	0	0
Cellular infiltration	0	0	0	0	0	0	1	0
Toxic neuropathy	0	0	0	0	0	0	1	0
Hyperplasia	0	0	0	0	0	0	0	1
LYMPH NODES	5	5	7	6	11	6	9	6
Reactive hyperplasia	1	1	2	2	0	0	2	4
Decomposition	0	0	1	0	0	0	0	0
Inflammation	1	0	0	0	2	2	3	1
KIDNEY	30	35	29	35	34	32	34	33
Nephropathy	17	25	10	0	13	14	12	16
Degeneration	0	0	0	2	1	1	3	4
Nephritis	1	0	18	22	2	5	0	1
Cell infiltrates	0	0	0	0	0	0	0	0
Vascular changes	1	0	1	4	0	0	0	1
Decomposition	0	1	0	0	0	1	0	0
SPLEEN	24	33	24	17	33	18	30	34
Amyloidosis	1	5	1	0	0	0	0	3
Vascular changes	11	12	5	7	13	1	10	15
Decomposition	1	3	1	0	0	0	0	2
Infarction	0	0	1	0	0	0	0	0
Degeneration	0	11	1	10	1	0	1	7
Fibrosis	0	0	0	1	0	0	0	0
Pigment accumulation	0	0	0	0	0	0	0	1
LIVER	27	35	24	35	34	29	34	33
Congestion	16	24	18	31	29	22	24	22
Amyloidosis	1	4	3	0	0	1	0	0
Degeneration	0	10	3	6	5	7	7	22
Fibrosis	0	0	0	1	0	0	0	0
Cellular infiltrates	0	0	7	1	0	1	0	0
Vascular changes	3	5	0	1	1	5	0	1
Bile duct dilation	3	2	0	0	0	0	3	0
Decomposition	0	2	0	0	0	0	3	5
Inflammation	0	1	0	0	0	0	1	0
Pigment accumulation	0	0	0	0	0	0	0	1

SUMMARY OF HISTOPATHOLOGY INCIDENCE TABLE 22

Group:	0.00%		0.04%		0.02%		0.01%	
	M	F	M	F	M	F	M	F
LUNGS	26	34	34	26	34	31	34	33
Inflammation	1	6	9	7	1	2	0	3
Emphysema	0	10	2	3	0	0	1	2
Degeneration	0	0	0	0	0	2	0	0
Vascular changes	19	25	25	19	37	33	35	24
Decomposition	0	0	0	0	0	1	0	0
Bronchiolar epith. hyperplasia	0	0	5	1	3	0	1	0
Alveolar cell metaplasia	1	11	4	0	2	1	1	8
INTESTINES	26	32	23	13	34	19	33	33
Decomposition	0	1	0	3	0	2	3	4
Inflammation	0	0	0	0	3	2	0	0
Epithelial atypia	0	0	0	0	0	1	0	0
Polyp	0	0	0	0	1	0	0	0
STOMACH	20	35	23	8	34	17	33	33
Vascular changes	0	0	0	0	1	0	0	0
Degeneration	0	0	0	0	1	2	0	1
Decomposition	0	2	0	0	0	2	3	3
Hyperkeratosis	0	1	0	0	0	0	0	0
ADRENAL GLAND	23	34	17	13	17	12	18	28
Amyloidosis	0	0	2	0	0	0	0	0
Vascular changes	2	8	3	2	3	4	6	17
Cyst	0	0	1	0	0	0	0	0
Inflammation	0	0	1	0	0	0	0	0
Degeneration	1	11	0	0	0	0	2	10
HEART	26	34	22	14	34	19	34	33
Thrombosis	0	5	1	3	2	1	1	6
Decomposition	0	0	1	0	0	0	3	4
Degeneration	3	0	1	2	2	0	5	10
Inflammation	4	4	0	0	0	0	1	5
TRACHEA	1	1	12	2	0	23	16	7
Desquamation	0	0	1	0	0	0	0	0
(Mycotic infection) candida	0	0	0	0	0	1	0	0
Squamous metaplasia	0	0	0	0	0	0	3	0
Calcification	0	0	0	0	0	0	0	1
ESOPHAGUS	0	0	2	2	0	0	0	0
URINARY BLADDER	20	29	18	6	32	10	27	10
Metaplasia	0	0	0	1	2	0	1	0
Decomposition	1	2	0	0	1	2	2	4
Epithelial hyperplasia	1	0	0	0	0	0	2	0
Epithelial atrophy	1	0	0	0	0	0	0	0

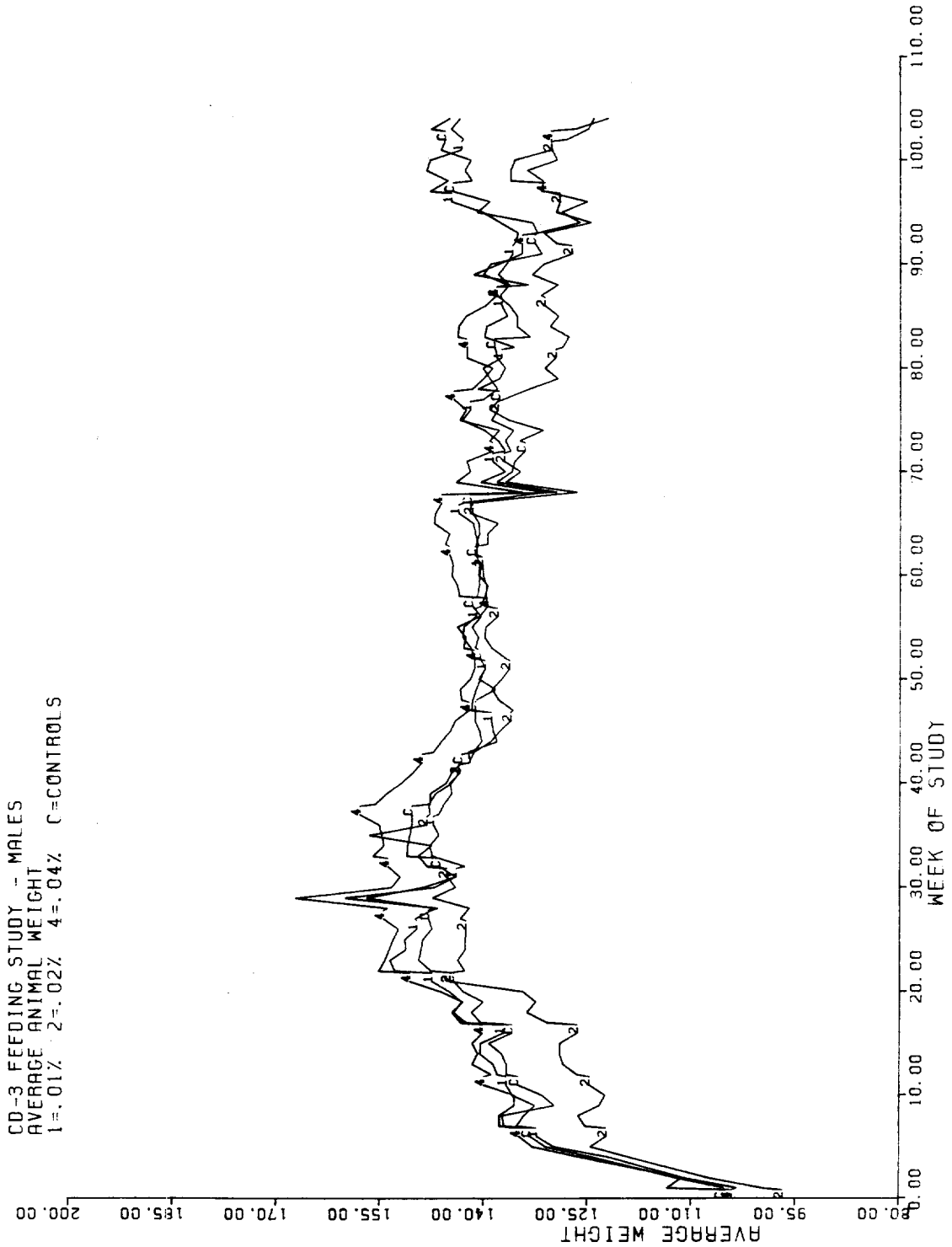
SUMMARY OF HISTOPATHOLOGY INCIDENCE TABLE 23

Group:	0.00%		0.04%		0.02%		0.01%	
	M	F	M	F	M	F	M	F
TESTIS	25	0	22	0	34	0	32	0
Atrophy	1	0	1	0	0	0	2	0
Nonspermatogenic epith.	0	0	0	0	0	0	1	0
PROSTATE	1	0	0	0	10	0	0	0
EPIDIDYMIS	1	0	0	0	10	0	0	0
Abscess	1	0	0	0	0	0	0	0
UTERUS	0	29	0	6	0	3	0	11
Squamous metaplasia	0	0	0	1	0	0	0	0
Decomposition	0	1	0	0	0	0	0	1
Papillary hyperplasia	0	1	0	0	0	0	0	0
Endometritis	0	0	0	0	0	0	0	1
OVARY	0	29	0	4	0	23	0	29
Cyst	0	0	0	1	0	2	0	0
Decomposition	0	1	0	0	0	1	0	1
Hemorrhage	0	0	0	0	0	1	0	0
Endometriosis	0	1	0	0	0	0	0	0
FALLOPIAN TUBES	0	17	0	0	0	3	0	11
Decomposition	0	1	0	0	0	0	0	1
SALIVARY GLAND	0	0	0	2	2	1	1	0
Edema	0	0	0	0	1	0	0	0
Hyperplasia	0	0	0	0	1	0	0	0
MUSCLE	0	1	0	0	0	0	0	0
Calcification	0	1	0	0	0	0	0	0
ADIPOSE TISSUE	0	0	0	0	0	0	0	2
Calcified nodule	0	0	0	0	0	0	0	1
GALLBLADDER	0	0	0	0	1	0	0	0
Stone	0	0	0	0	1	0	0	0
STERNUM	0	0	17	2	0	0	0	0
BRAIN	25	31	23	13	33	13	29	32
Decomposition	0	0	0	0	0	0	0	0
Edema	0	0	0	1	0	0	0	0
BONE MARROW	23	31	5	23	10	7	32	29
Decomposition	0	0	0	0	0	0	0	0
Hypoplasia	0	0	0	4	0	0	4	0
Bloody	0	0	0	0	0	0	0	0
Fibrotic	0	0	0	0	0	0	0	0

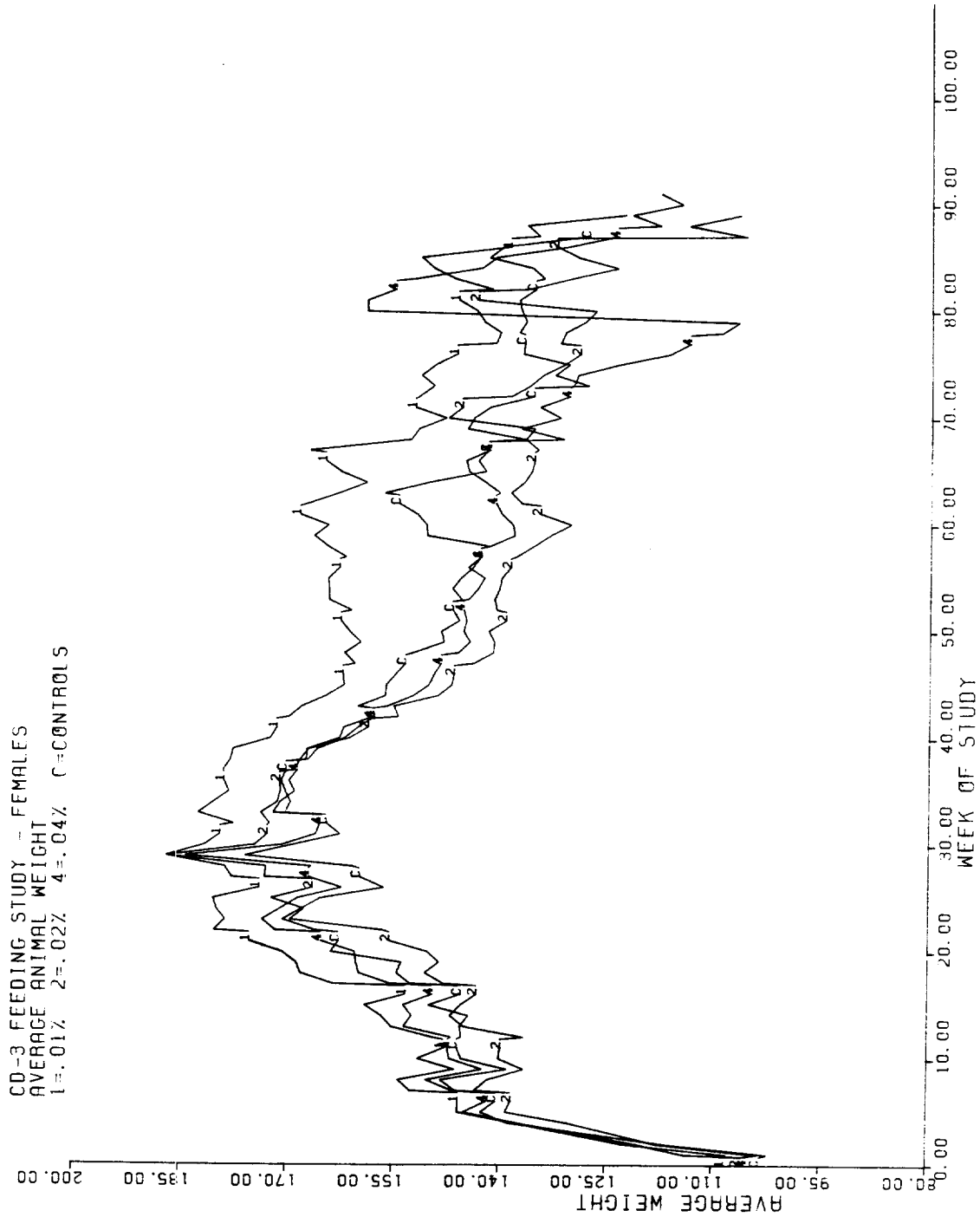
SUMMARY OF HISTOPATHOLOGY INCIDENCE TABLE 24

Group:	0.00%		0.04%		0.02%		0.01%	
	M	F	M	F	M	F	M	F
PANCREAS	6	27	11	11	7	11	8	8
Decomposition	0	1	1	0	0	0	0	0
THYROID GLAND	11	25	10	23	30	31	26	23
Degeneration	1	0	1	1	1	1	0	0
Hyperplasia	0	0	0	1	0	0	0	0
Amyloidosis	0	0	0	0	1	0	0	0

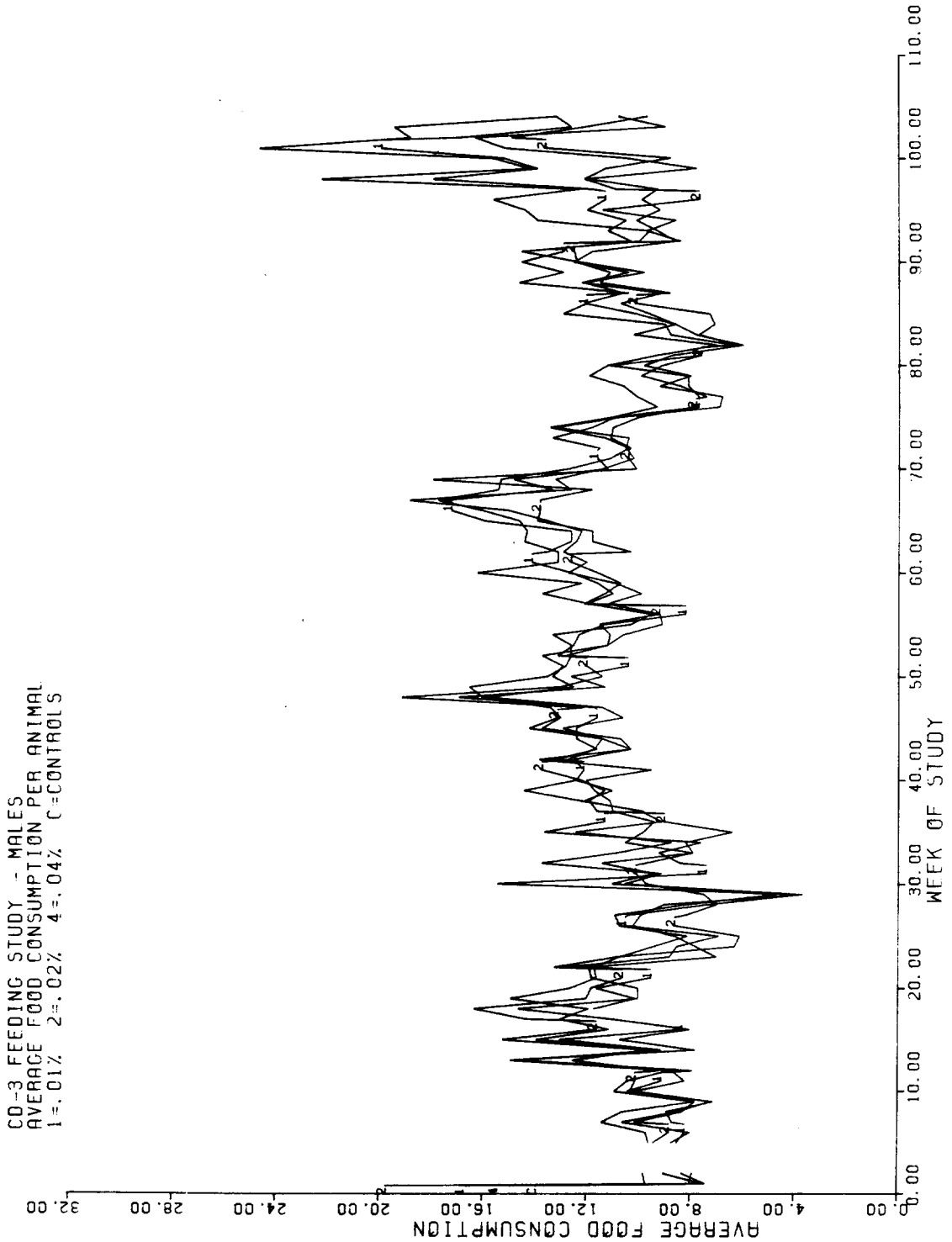
Graph 1


CD-3 FEEDING STUDY - MALES
AVERAGE ANIMAL WEIGHT
1=.01% 2=.02% 4=.04% C=CONTROLS
AVERAGE WEIGHT
80.00
95.00
110.00
125.00
140.00
155.00
170.00
185.00
200.00
WEEK OF STUDY
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00
110.00

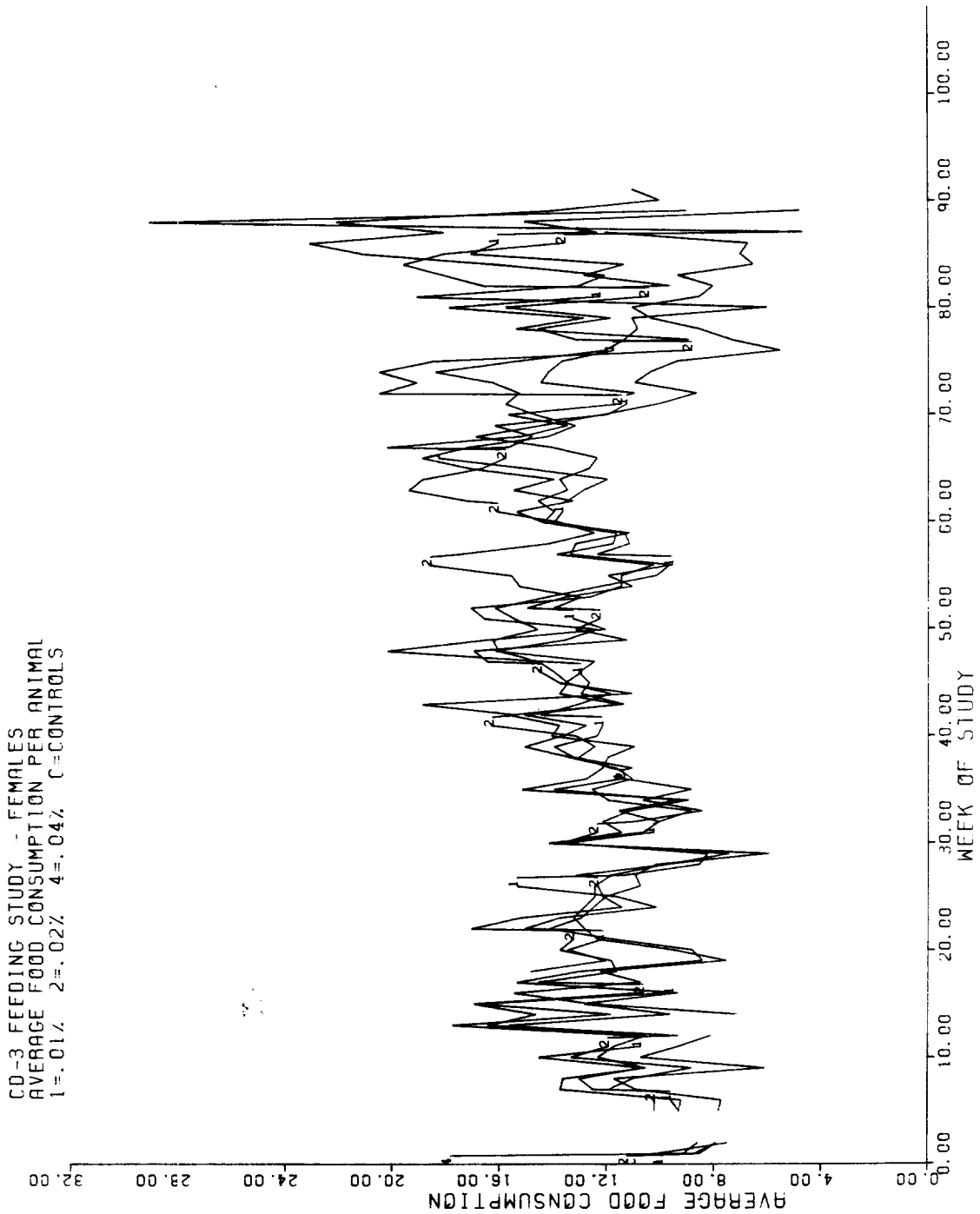
Graph 2


CD-3 FEEDING STUDY - FEMALES
AVERAGE ANIMAL WEIGHT
1=.01% 2=.02% 4=.04% C=CONTROLS
AVERAGE WEIGHT
80.00
95.00
110.00
125.00
140.00
155.00
170.00
185.00
200.00
WEEK OF STUDY
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00

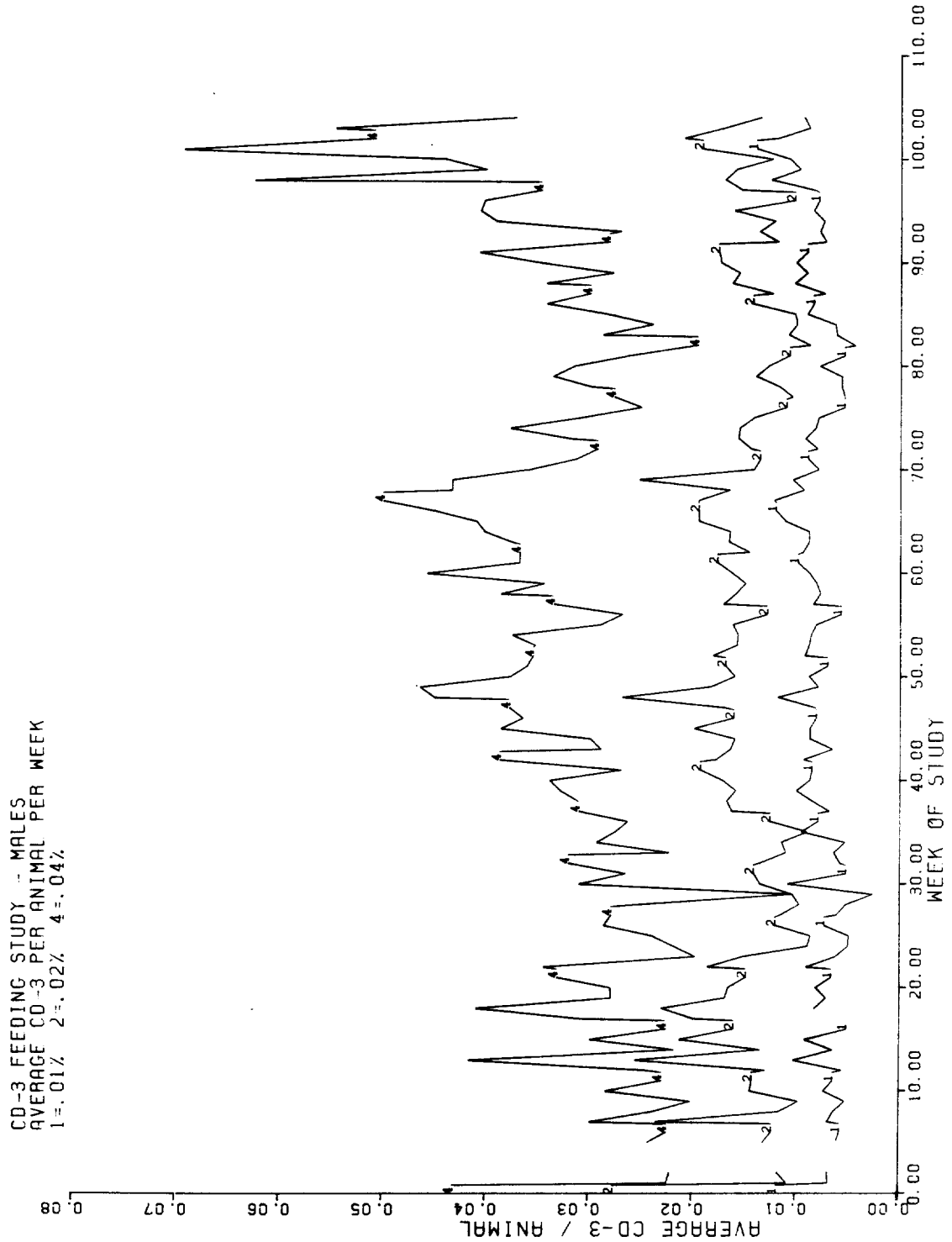
Graph 3


CD-3 FEEDING STUDY - MALES
AVERAGE FOOD CONSUMPTION PER ANIMAL
1=.01% 2=.02% 4=.04% C=CONTROLS
AVERAGE FOOD CONSUMPTION
0.00
4.00
8.00
12.00
16.00
20.00
24.00
28.00
32.00
WEEK OF STUDY
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00
110.00

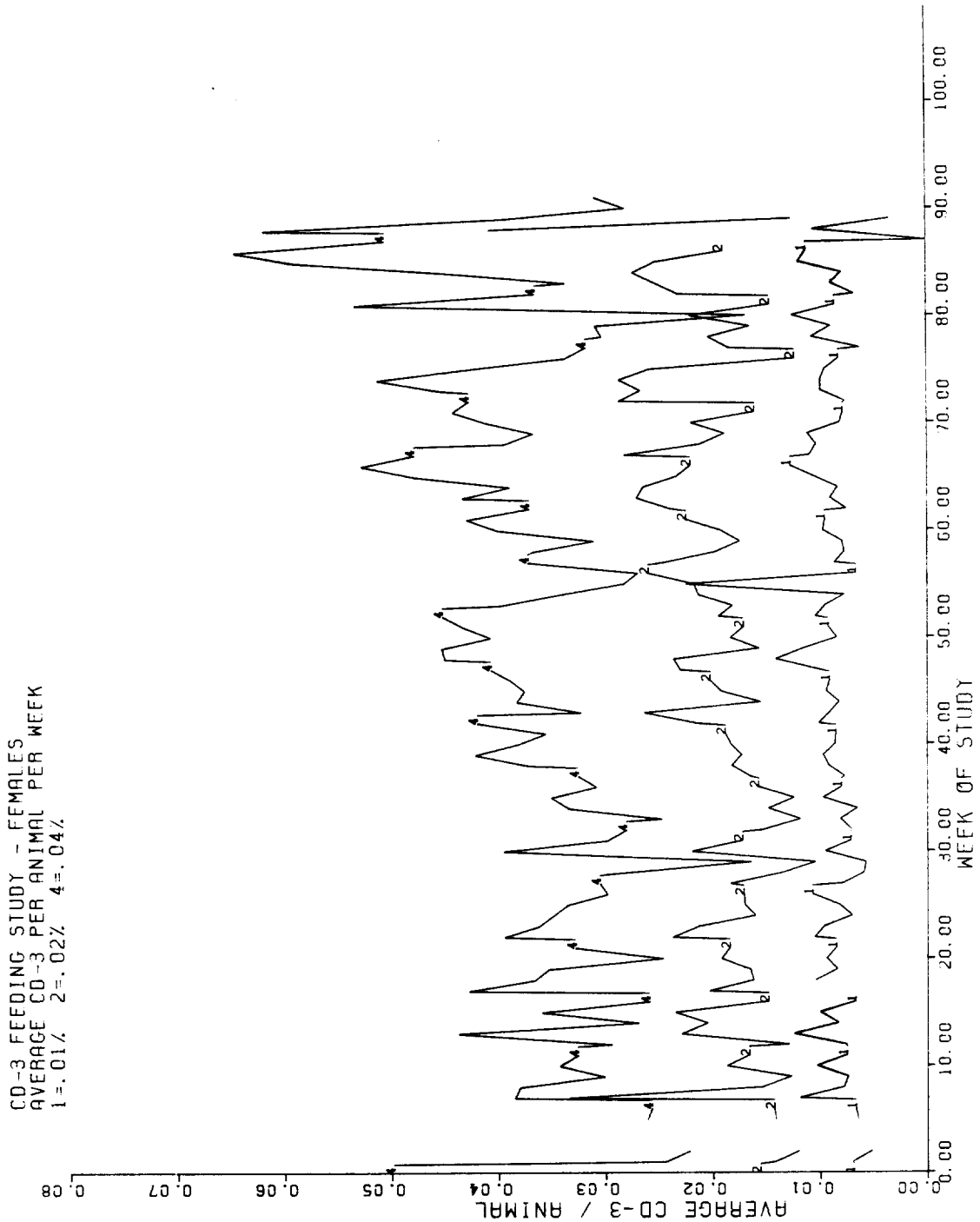
Graph 4


CD-3 FEEDING STUDY - FEMALES
AVERAGE FOOD CONSUMPTION PER ANIMAL
1=.01% 2=.02% 4=.04% C=CONTROLS
AVERAGE FOOD CONSUMPTION
0.00
4.00
8.00
12.00
16.00
20.00
24.00
28.00
32.00
WEEK OF STUDY
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00

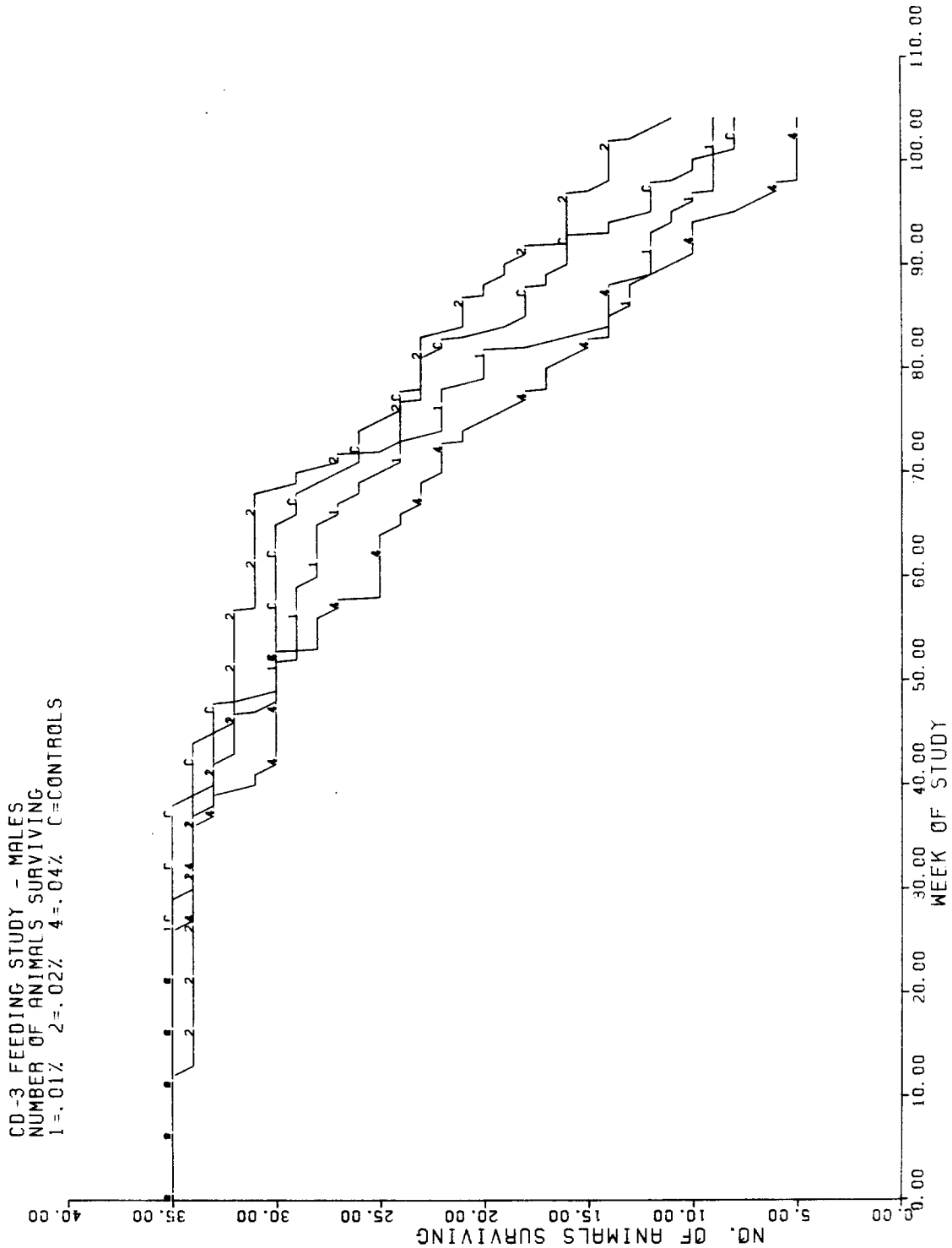
Graph 5


CD-3 FEEDING STUDY - MALES
AVERAGE CD-3 PER ANIMAL PER WEEK
1=.01% 2=.02% 4=.04%
AVERAGE CD-3 / ANIMAL
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
WEEK OF STUDY
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00
110.00

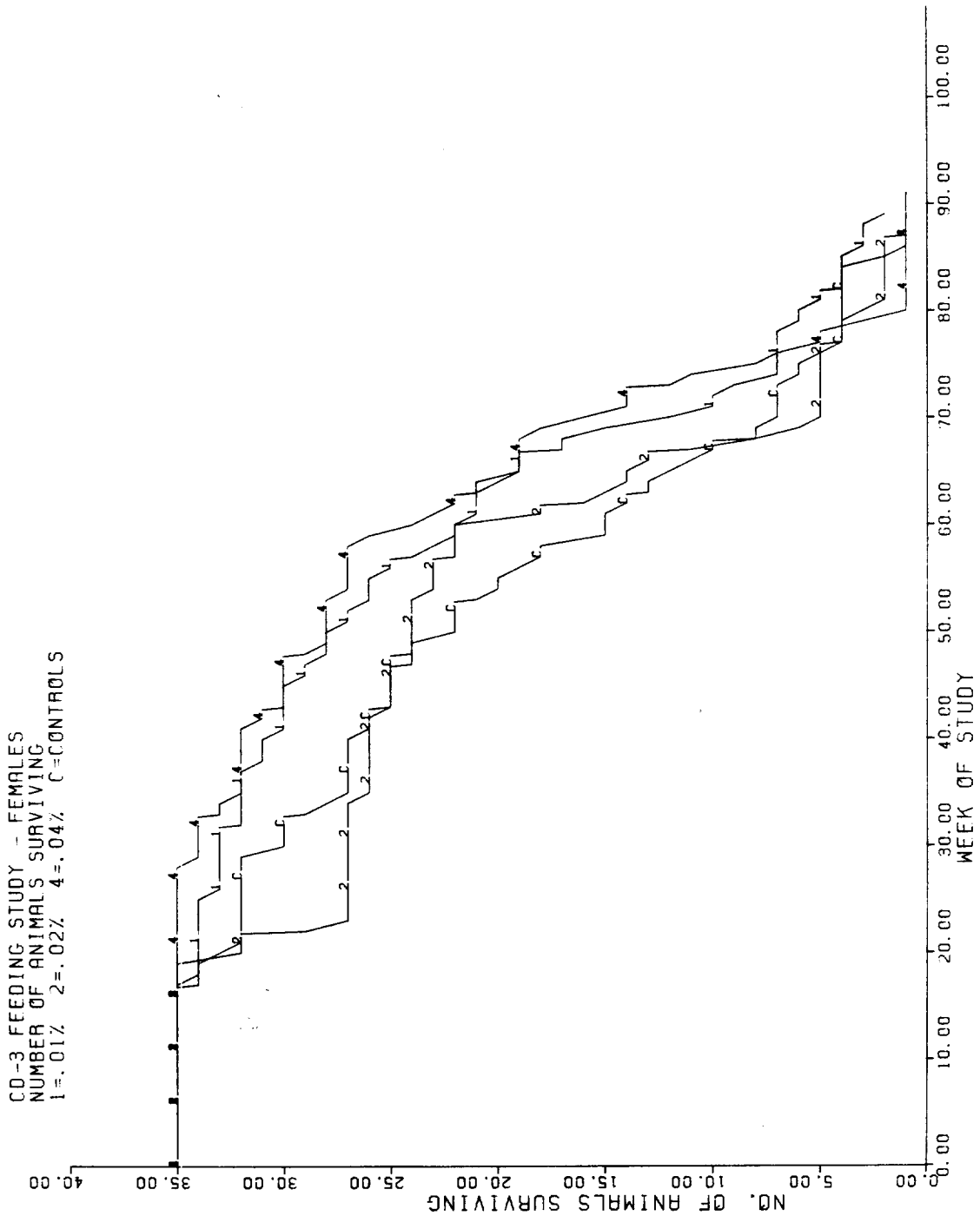
Graph 6


CD-3 FEEDING STUDY - FEMALES
AVERAGE CD-3 PER ANIMAL PER WEEK
1=.01% 2=.02% 4=.04%
AVERAGE CD-3 / ANIMAL
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
WEEK OF STUDY
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00

Graph 7


CD-3 FEEDING STUDY - MALES
NUMBER OF ANIMALS SURVIVING
1=.01% 2=.02% 4=.04% C=CONTROLS
NO. OF ANIMALS SURVIVING
WEEK OF STUDY
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00
110.00

Graph 8


CD-3 FEEDING STUDY - FEMALES
NUMBER OF ANIMALS SURVIVING
1=.01% 2=.02% 4=.04% C=CONTROLS
NO. OF ANIMALS SURVIVING
WEEK OF STUDY
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
0.00
10.00
20.00
30.00
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Milan S. Vlaovic, D.V.M., Ph.D.
Toxicology Section

Approved:

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Mouse Skin Painting Study of Color Developer CD-3

Toxicology Section

W.J. Krasavage, M.S.

March 22, 1981

134739J
TX-81-50

Mouse Skin Painting Study of Color Developer CD-3

Introduction

Color developer CD-3 was tested for potential carcinogenicity using a lifetime skin painting technique in mice. The study was conducted by the Toxicology Section of the Health, Safety, and Human Factors Laboratory, Eastman Kodak Company, Rochester, N.Y.

Materials and Methods

Animals:

One hundred ninety-two female CF-1 mice (Carworth Farms, Inc., New City, New York) were randomly assigned to three groups of 64 animals each. They were housed eight per cage in polycarbonate cages. Aspen wood chips were used for bedding and Purina Rat Chow(R) and drinking water were available ad libitum. Mice within each cage were identified by toe-clipping and the backs of the animals were clipped free of hair with Oster electric clippers prior to beginning the study and on an "as needed" basis thereafter.

Compounds and Doses:

Color developer CD-3 (EKC Accession No. 905401; HS&HFL No. 73-344) was received as a 5% solution of the sulfate. The Biochemistry Section of

Health, Safety, and Human Factors Laboratory prepared the solution by adding 5.75 g of CD-3 to a beaker containing 50 mL of distilled water. This was heated on a steam bath until completely dissolved, then transferred to a graduated cylinder. The beaker was rinsed several times with 2-(2-ethoxy-ethoxy)ethanol (2EEE). The rinses were added to the cylinder and the volume was adjusted to 114 mL with 2EEE. The solution was transferred to a 120 mL amber bottle and 1.1 mL of Triton X-100 was added. New solutions were prepared and sent to the Toxicology Section every two weeks.

Prior to the application to the backs of the mice, the salt solution was converted to the free base by adding 0.023 mL of concentrated NH_4OH (14.8 M, 28% NH_3) per mL of salt solution. The free base is the form used in the photographic process. This conversion provided a 3.1% solution of the free base. A quantity of 0.05 mL of this solution was applied to the backs of the mice three times weekly over a 772-day period using a calibrated automatic pipette (Biopette$^{(R)}$, Lab Cages Inc.). This treatment provided doses of 1.55 mg of CD-3 as the free base per mouse or approximately 62 mg/kg per application.

Negative control animals received 0.05 mL of the solvent 2-(2-ethoxy-ethoxy)ethanol three times weekly over an 881-day period. This provided doses of 50 mg per application or 2000 mg/kg.

The positive control mice received 0.05 mL of a 0.3% solution of benzo [α]pyrene in 2 EEE; three times weekly over a 180-day period. The dose per application was 0.15 mg or 6 mg/kg.

Clinical and Pathologic Examinations:

Individual body weights were recorded prior to beginning treatment, weekly during the first two months and monthly for the next five months.

Clinical observations were made five days per week. Mortality, the time to appearance and the description of any masses were recorded.

Moribund animals and survivors at termination were killed by inhalation of CO_2 or ether and examined for evidence of gross pathology. Tissues from these animals and those dying spontaneously were fixed in 10% buffered formalin, processed for paraffin embedment, stained with hematoxylin-eosin, and examined by light microscopy. All tumors or masses from the application site present at the time of autopsy were examined histologically. Wherever possible tissues from other organs were also examined. Tissues from animals dying spontaneously were frequently not available either because of cannibalism or autolysis.

Statistical Methods:

Survival was analyzed using a life table procedure in "Statistical Package for the Social Sciences" in SPSS Update, New Procedures and Facilities for Release 7 and 8, McGraw Hill Book Company, 1979. Student's "t" test was used to compare the mean time to appearance of the subcutaneous masses.

Results

Survival:

Fifty-seven CD-3 mice died spontaneously during a 772-day treatment period and seven animals were sacrificed moribund (Table 1).

Forty eight negative control mice died spontaneously over an 881-day period, 10 mice were sacrificed moribund and six survivors were killed at termination.

Two positive control animals died spontaneously; one on day 175 and one on day 177 of the study. The other 62 animals developed tumors and were killed; 47 on day 177 and 15 on day 180.

The cumulative probability of survival of the CD-3 and negative control mice is presented in Figure 1. The expected survival of the CD-3 mice was statistically significantly less than the negative control animals ($p \leq .0025$). The median life span was 572.4 days and 657.6 days for the CD-3 and negative control groups, respectively.

Body Weights and Clinical Signs:

None of the treatments had a significant effect on body weight gain (Figure 2). No unusual signs of toxicity were noted throughout the study.

Pathology:

Positive Controls:

All positive control mice developed tumors in the treated area typical of those produced by benzo[α]pyrene. The first one appeared on day 94 of the study and all animals had one or more tumors by day 180. The group was terminated at this time (Table 1).

Histologic examination of skin from the treated area revealed 58 sections with squamous cell carcinomas and one with a keratoacanthoma. Pulmonary metastases of a squamous cell carcinoma was found in one mouse, and one animal had a thymic lymphoreticular neoplasm.

CD-3 Group:

What appeared to be a small (1 mm) papilloma was found in the middle of the lower back of one CD-3 mouse on day 98 of the study. The mass remained the same size and was not present when this animal was examined on day 141. The animal died on the 455th day and no lesions were noted on autopsy.

Five of the CD-3 mice each developed a subcutaneous mass at a site other than the treated area. The median time to the appearance of these masses was 497 days with a range of 427-528 days. The time to appearance of the first mass was not statistically significant compared to the solvent control for a comparable period (i.e. 772 days).

Histologic examination of the tissues from all but five of the CD-3 mice was precluded because the majority of the samples were lost during shipment to a private pathology laboratory; thus none of the subcutaneous masses were examined histologically.

No masses had been found in the treated area on any of the CD-3 animals at termination.

Negative Control:

One negative control mouse had a small mass in the treated area which appeared on day 669 of the study. The size (1-2 mm) of the mass remained the same until the animal died four months later. Histologic examination revealed that this mass was a papilloma.

Ten negative control mice developed 15 subcutaneous masses at sites other than the treated area over a 772-day period. The median time to appearance of the first tumor per mouse was 605 days with a range of

408-748 days. Two other negative control mice developed three subcutaneous masses (774, 779 and 835 days) before the group was terminated on day 881.

Histologically, the masses were diagnosed as osteoma, osteosarcoma, fibrosarcoma, carcinoma (axillae), mammary adenocarcinoma or lymphoreticular neoplasm.

Summary

Sixty-two mg/kg of CD-3 applied as the free base to the clipped backs of mice three times weekly over a 772-day period did not produce malignant tumors in the treated area. One animal had a papilloma which persisted for 41 days, did not increase in size and disappeared.

The negative control mice received 2000 mg/kg of the solvent 2-(2-ethoxy-ethoxy)ethanol three times weekly over an 881-day period. No malignant tumors were found in the treated area. One mouse had a papilloma which appeared in the treated area on day 669 of the study.

Subcutaneous masses at sites removed from the treated area were more prevalent (15 vs 6) in the negative control mice than in the CD-3 animals for a comparable period of time (772 days).

The CD-3 animals had a significant lower probability of survival compared to the negative control. Median life span was 572.4 and 657.6 days, respectively.

None of the treatments appeared to have an adverse affect on body weight gain.

WJK:ewe
3/22/82

Table 1: Color Developer CD-3 Cancer Study

Group	No. of Spontaneous Deaths	No. of Mice Sacrificed Moribund	No. Mice Killed At Term	No. Masses in Treated Area	No. Mice with Masses in Treated Area	Appearance 1st Mass (Days $\bar{x}$±SD)
CD-3	57	7	0	1[b]	1	98
Benzo[α]pyrene	2	0	62	>221	64[d]	125.2±24.1
Negative control	48	10	6	1[c]	1	669

a = Survival of CD-3 animals was significantly less than controls (see Figure 1).

b = Appeared to be a papilloma; was absent 43 days later.

c = Diagnosed histologically as a papilloma.

d = Of 62 skin sections examined 58 showed squamous cell carcinomas.

Figure 1

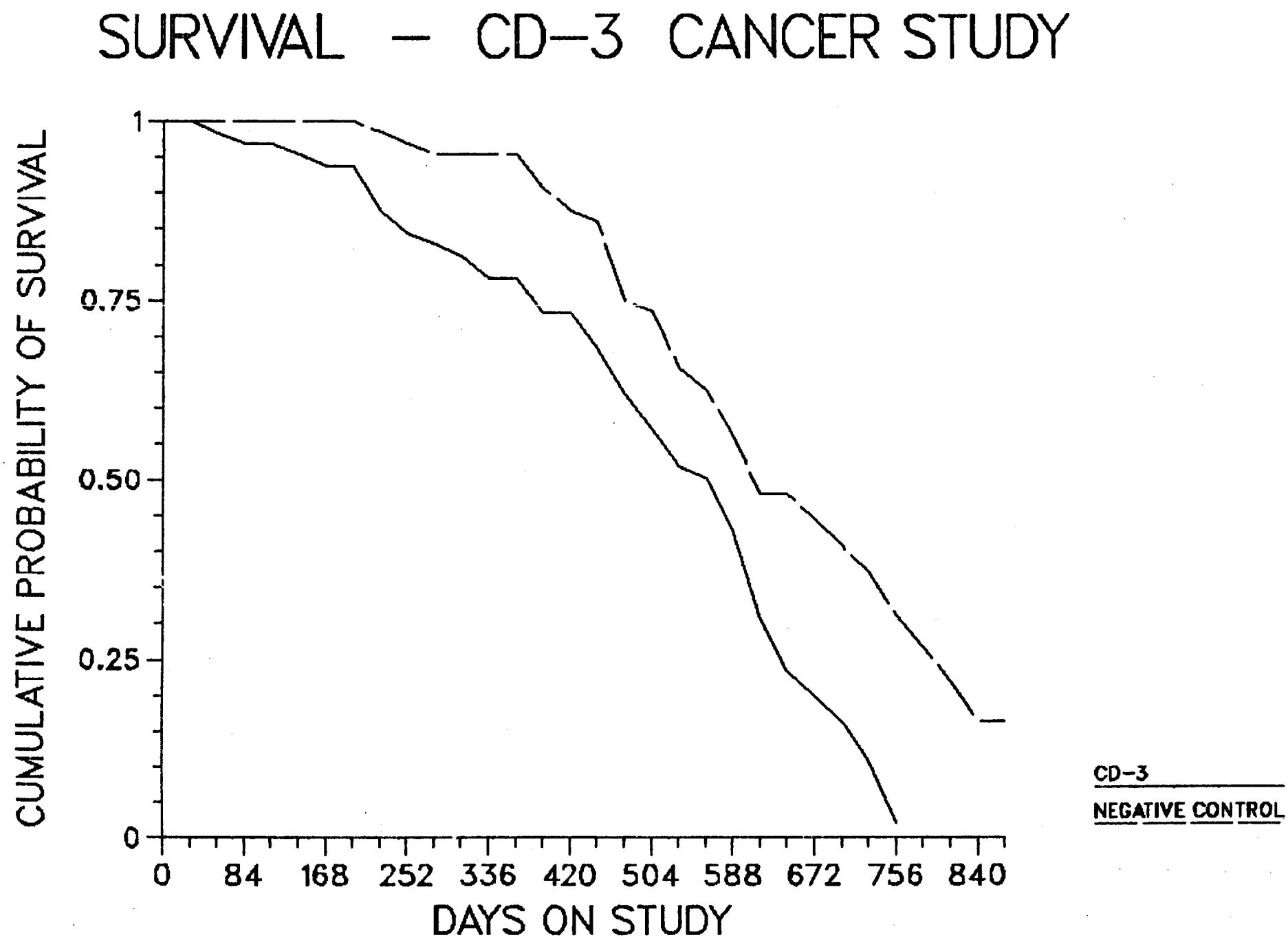
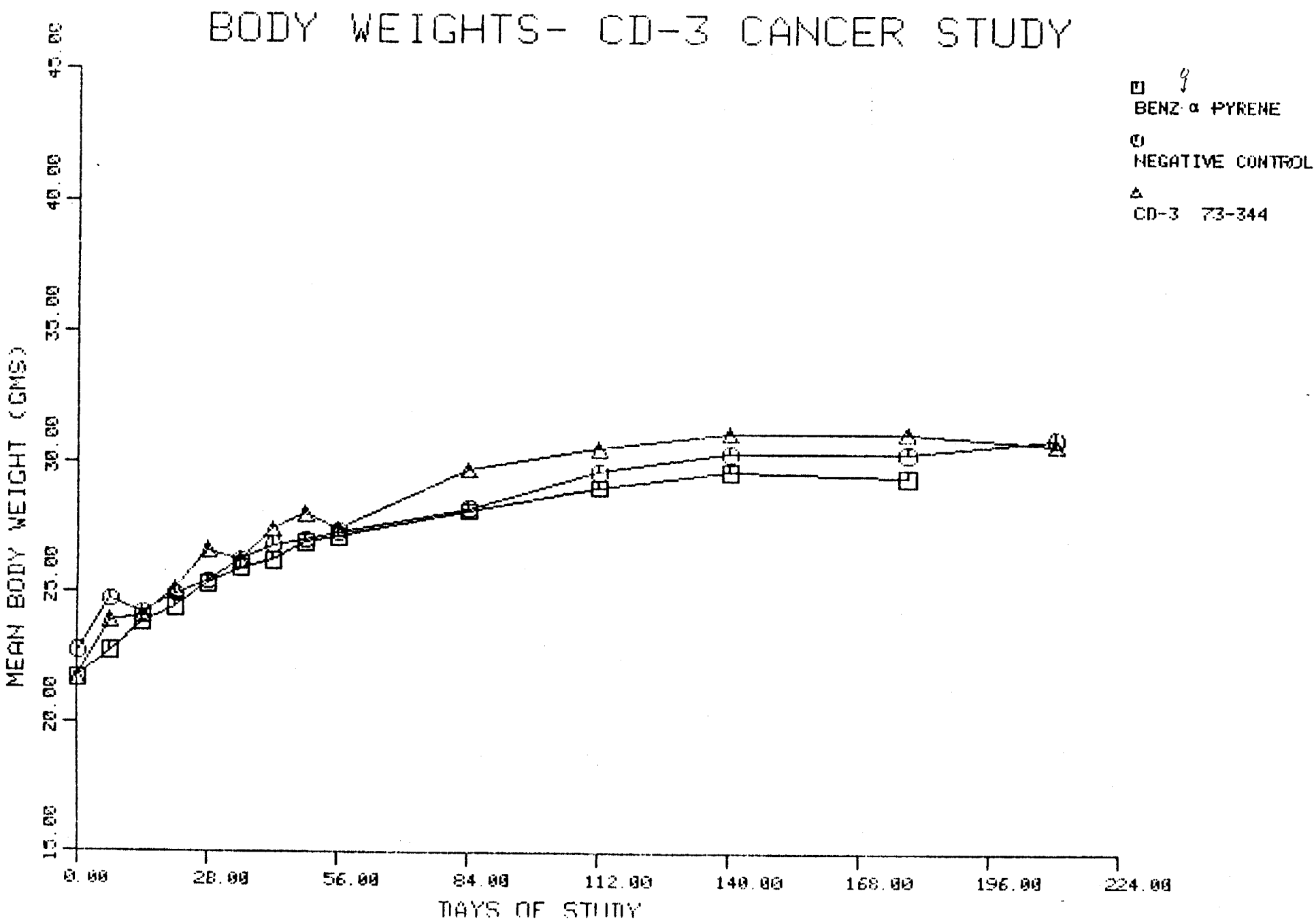

SURVIVAL – CD-3 CANCER STUDY
CUMULATIVE PROBABILITY OF SURVIVAL
1
0.75
0.50
0.25
0
0
84
168
252
336
420
504
588
672
756
840
DAYS ON STUDY
CD-3
NEGATIVE CONTROL

Figure 2


BODY WEIGHTS- CD-3 CANCER STUDY
BENZ α PYRENE
NEGATIVE CONTROL
CD-3 73-344
MEAN BODY WEIGHT (GMS)
45.00
40.00
35.00
30.00
25.00
20.00
15.00
0.00
28.00
56.00
84.00
112.00
140.00
168.00
196.00
224.00
DAYS OF STUDY

Walter J. Krasavage

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Toxicology Section

Approved:

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C.J. Terhaar, Ph.D.
Supervisor, Toxicology Section

LBI PROJECT #2547

90540/

MUTAGENIC EVALUATION OF

COMPOUNS 75-147 (CD3)

SUBMITTED TO

EASTMAN KODAK COMPANY
1669 LAKE AVENUE
ROCHESTER, NEW YORK 14650

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

JULY 18, 1975

Litton BIONETICS

DATE: July 18, 1975

SPONSOR: Eastman Kodak, Rochester, New York

SUBJECT: Plate test evaluation of test compound, 75-147 (CD3)

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: June 27, 1975
2. Description: Beige crystals

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strain TA-1535
strain TA-1537
strain TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests: (components 1-4 make up the "Core Reaction Mixture"):

Component	Final Concentration/ml
1. TPN (sodium salt)	6 μM
2. Isocitric acid	49 μM
3. Tris buffer, pH 7.4	28 μM
4. $MgCl_2$	1.7 μM
5. Supernatant fraction equivalent to 70 mg of fresh tissue	

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D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from liver, lung, kidney tissues of the following mammalian species: Mice-Flow ICR random bred males, Rats-Sprague-Dawley males, Monkeys-rhesus (Macacca mulatta) males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Ethyl methanesulfonate (EMS)	Water or saline	BPS[b]
	2-Nitrofluorene (NF)	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard (QM)	Water or saline	FS[b]
Activation	Dimethylnitrosamine (DMNA)	Water or saline	BPS[b]
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide[c]	FS[b]
	7,12-Dimethylbenzanthracene (DMBA)	Dimethysulfoxide[c]	FS[b]

a Concentrations given in the Results Section
b BPS = base-pair substitution; FS = frameshift
c Previously shown to be non-mutagenic

III. METHODS

A. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities of tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor driven homogenizing unit at 4C. The whole organ homogenate obtained from this step was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80C. Samples from these preparations were used for the activation studies.

B. Plate Tests (Spot Test)

Approximately 10^9 cells from a log phase culture of each indicator strain was added to test tubes containing 2.0 ml of molten agar supplemental with biotin and a trace of histidine. For nonactivation tests, the contents of the appropriate tubes were poured over the surfaces of selective agar plates. In activation tests, the 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. The contents of each tube were poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. Samples of the test chemical were then placed on the surface of the overlay agar containing the indicator system. The plates were incubated for 48-72 hours at 37C and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds which are active directly and those which require metabolic activation were run with each assay.

C. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

B. Plate Tests (Spot Test)

Approximately 10^9 cells from a log phase culture of each indicator strain was added to test tubes containing 2.0 ml of molten agar supplemental with biotin and a trace of histidine. For nonactivation tests, the contents of the appropriate tubes were poured over the surfaces of selective agar plates. In activation tests, the 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. The contents of each tube were poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. Samples of the test chemical were then placed on the surface of the overlay agar containing the indicator system. The plates were incubated for 48-72 hours at 37C and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds which are active directly and those which require metabolic activation were run with each assay.

C. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

IV. SUMMARY OF TEST RESULTS

Plate Tests

A. Name or code designation of the test compound: 75-147 (CD3)

B. Test date: July 14, 1975

C. Concentration of the test compound: 50 µg/plate

Test	Species	Tissue	REVERTANTS/PLATE TA-1535 1	TA-1535 2	TA-1537 1	TA-1537 2	TA-1538 1	TA-1538 2	D4 1
1. Non-activation									
Solvent Control	---	---	5		10		12		31
Positive Control[a]	---	---	>10^4		436		>500		214
Test Compound	---	---	5	9	11	7	19	17	28
2. Activation									
Negative Control	---	---	18		23		20		22
Solvent Control	---	---	23		13		36		12
Reaction Mixture Control	---	---	10		11		18		17
Positive Control[b]	Mouse	Liver	151		46		163		--
Positive Control		Lung	25		30		22		--
Positive Control		Kidney	11		28		19		--
Positive Control	Rat	Liver	102		33		189		--
Positive Control		Lung	17		25		27		--
Positive Control		Kidney	29		2		30		--
Positive Control	Monkey	Liver	115		38		110		--
Positive Control		Lung	16		19		19		--
Positive Control		Kidney	24		20		23		--
Test Compound	Mouse	Liver	11	9	15	21	10	17	22
Test Compound		Lung	5	8	13	21	17	16	26
Test Compound		Kidney	7	8	11	18	10	12	21
Test Compound	Rat	Liver	8	13	14	16	15	11	22
Test Compound		Lung	10	11	15	20	13	13	25
Test Compound		Kidney	10	7	23	17	19	23	17
Test Compound	Monkey	Liver	12	9	14	15	12	15	19
Test Compound		Lung	12	10	10	26	13	11	16
Test Compound		Kidney	16	6	18	15	15	14	28

a

TA-1535	EMS	10 µl/plate
TA-1537	QM	20 µg/plate
TA-1538	NF	100 µg/plate
D-4	EMS	10 µl/plate

b

TA-1535	DMNA	50 µM/plate
TA-1537	AAF	100 µg/plate
TA-1538	AAF	100 µg/plate
D-4	DMNA	100 µM/plate

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V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test chemical, 75-147 (CD3), was evaluated for genetic activity in a series of plate assays using microbial cells as indicators of mutagenic or recombinogenic activity. The assays were conducted so that each compound was evaluated directly and in the presence of *in vitro* mammalian metabolic activation. The following results were obtained:

A. Nonactivation Assays

The results of all nonactivation plate tests were negative for this test compound. All control assays were satisfactory.

B. Activation Assays

The test compound was evaluated for mutagenic activity in the presence of metabolic activation from mouse, rat and rhesus monkey tissues. The mouse and rat tissues were from animals previously induced with Arochlor 1254 to enhance microsomal enzyme content in the activation preparations. The results of the assays were negative. The controls were satisfactory.

C. Conclusions

Test Compound, 75-147 (CD3), was not mutagenic for the bacterial indicator organisms or recombinogenic for the yeast indicator organism under the test conditions employed in this evaluation.

Submitted by:

Robert Weir for
David Brusick, Ph.D.
Director of Genetics

Litton BIONETICS

903478 905401 904984

INDUSTRIAL LABORATORY REPORT for Environmental Sciences Group--HS & HFL

Requested by: Mr. W. Ewell

Date Received: 4/18/83 through 4/21/83

Submitting Department Reference

Industrial Laboratory File Nos. FE3H010 Date 6/23/83 Dept. Charge No. EWO No. 700-7721

TITLE: CD-3 Photodegradation Study

Author: Karen Burgess

Samples: (Labels and Description) 50 mg/l CD-3, X1, X2, X3, X4, F1, F2, F3, F4, C4 (control)

Abstract: High Performance Liquid Chromatography (HPLC) and a colorimetric procedure for hydroxylated aromatic compounds was used to analyze aqueous solutions of CD-3 in support of a photodegradation study. Samples were analyzed at time zero, various times during the first eight hours, 24 hrs., 48 hrs., and 72 hrs.

For additional information or further work contact Karen Burgess, Rick Schumacher

Report: Samples were analyzed by HPLC for the presence of CD-3 over a 72 hour period. Table I lists the HPLC parameters used to analyze for CD-3. The resulting data, consisting of sampling time and concentration of CD-3 for test and control samples are listed in Table II. Using this data, the half-life for X4 and F4 was determined to be 3.1 hrs. ($k = -0.22\ hr^{-1}$) and 9.9 hrs. ($k = -0.06\ hr^{-1}$), respectively.

For C4 (control), the concentration of CD-3 is reported as none detected. The detection limit is the lowest amount of CD-3 which the HPLC could measure in a "clean" matrix. For other test solutions, the concentration of CD-3 is reported as not quantifiable. The quantifiable limit is higher than the detection limit in some cases due to the lack of baseline resolution caused by other components in the sample.

Figures 1 and 2 show the plots of the data for the test solutions. Data for the X4 appears to deviate somewhat from first order kinetics. This may be due to the warming effects caused by the xenon arc lamp.

A colorimetric procedure for measuring hydroxylated aromatic compounds (Tannin-Lignin, Method 513, APHA, 15th ed., p. 536) was used as an additional indicator of sample degradation. The data, listed in Table III, indicates that there was little difference between samples exposed to xenon arc light or fluorescent light.

Additional data on following 5 pages.

Signed: Karen F. Burgess

KP 605608

Table I

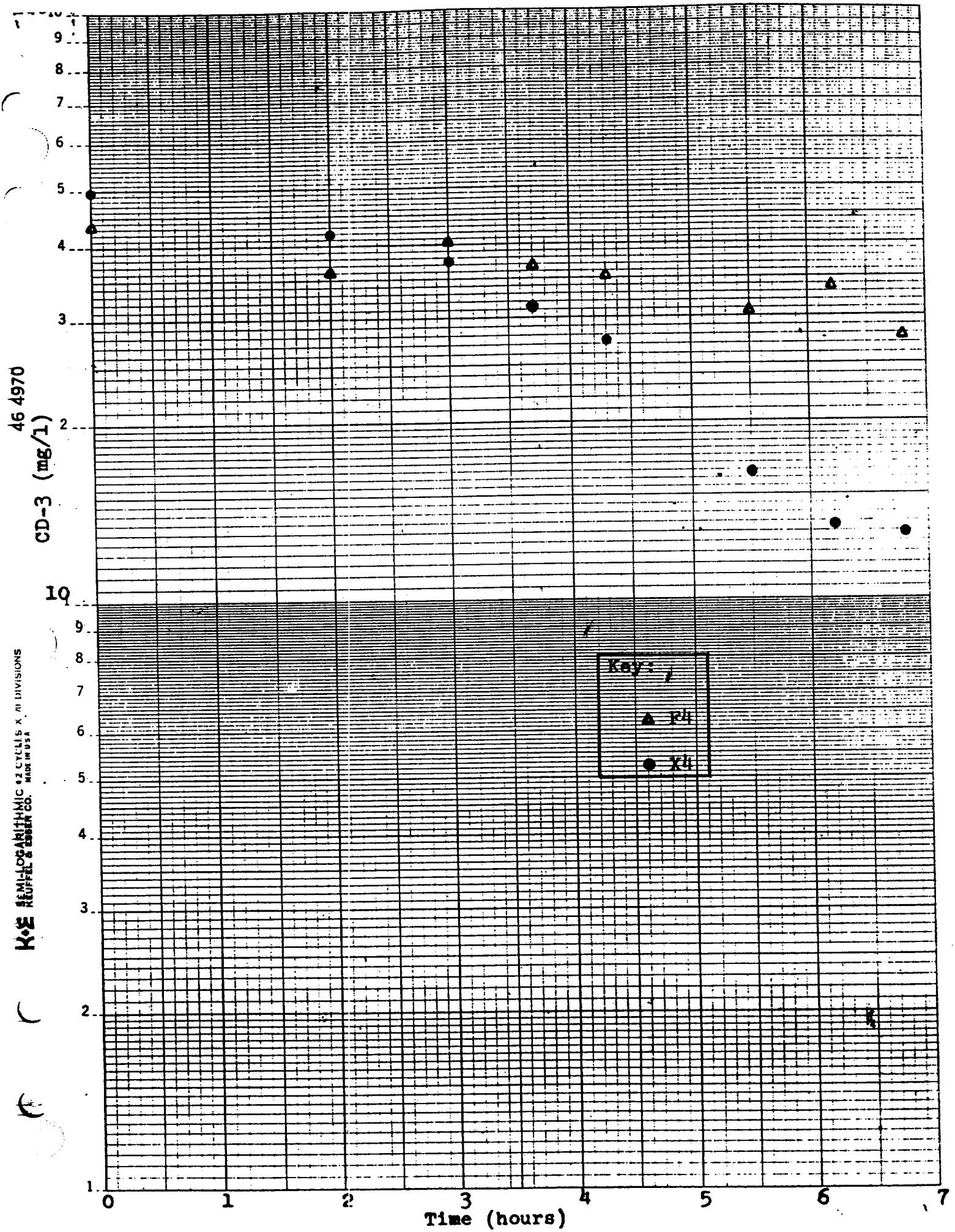
HPLC parameters--

Column: Waters uBondapak RP-18, 30cm x 3.9 mm
Mobile phase: Isocratic
50 % HPLC grade methanol
50 % PIC reagent B-6
Flow: 1 ml/min
Detector: UV, 238 nm @ 0.04 aufs
Sample size: 20 ul

Table II

Sampling Time (hrs.)	Conc. (ppm) C4	X1	X2	X3	X4	F1	F2	F3	F4
0	ND	50.3	50.7	50.4	49.2	47.8	43.4	46.0	43.2
2	ND	NA	NA	NA	41.9	NA	NA	NA	36.0
3	ND	NA	NA	NA	37.4	NA	NA	NA	40.2
3.7	ND	NA	NA	NA	31.2	NA	NA	NA	36.9
4.3	ND	NA	NA	NA	27.3	NA	NA	NA	35.1
5.5	ND	NA	NA	NA	16.3	NA	NA	NA	30.6
6.2	ND	NA	NA	NA	13.3	NA	NA	NA	33.5
6.8	ND	NA	NA	NA	12.9	NA	NA	NA	27.8
24	ND	NA	NQ	NQ	NQ	NA	7.0	6.0	7.4
48	ND	NA	NA	NQ	NQ	NA	NA	NQ	NQ
72	ND	NA	NA	NA	NQ	NA	NA	NA	NQ

ND = none detected (lower limit of detection ~ 0.5 mg/l)
NQ = not quantifiable (limit of quantitation = 4 ppm due to interference from degradation products)
NA = not analyzed


46 4970
K+E SEMI-LOGARITHMIC 62 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
CD-3 (mg/1)
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
Time (hours)
Key:
r4
x4

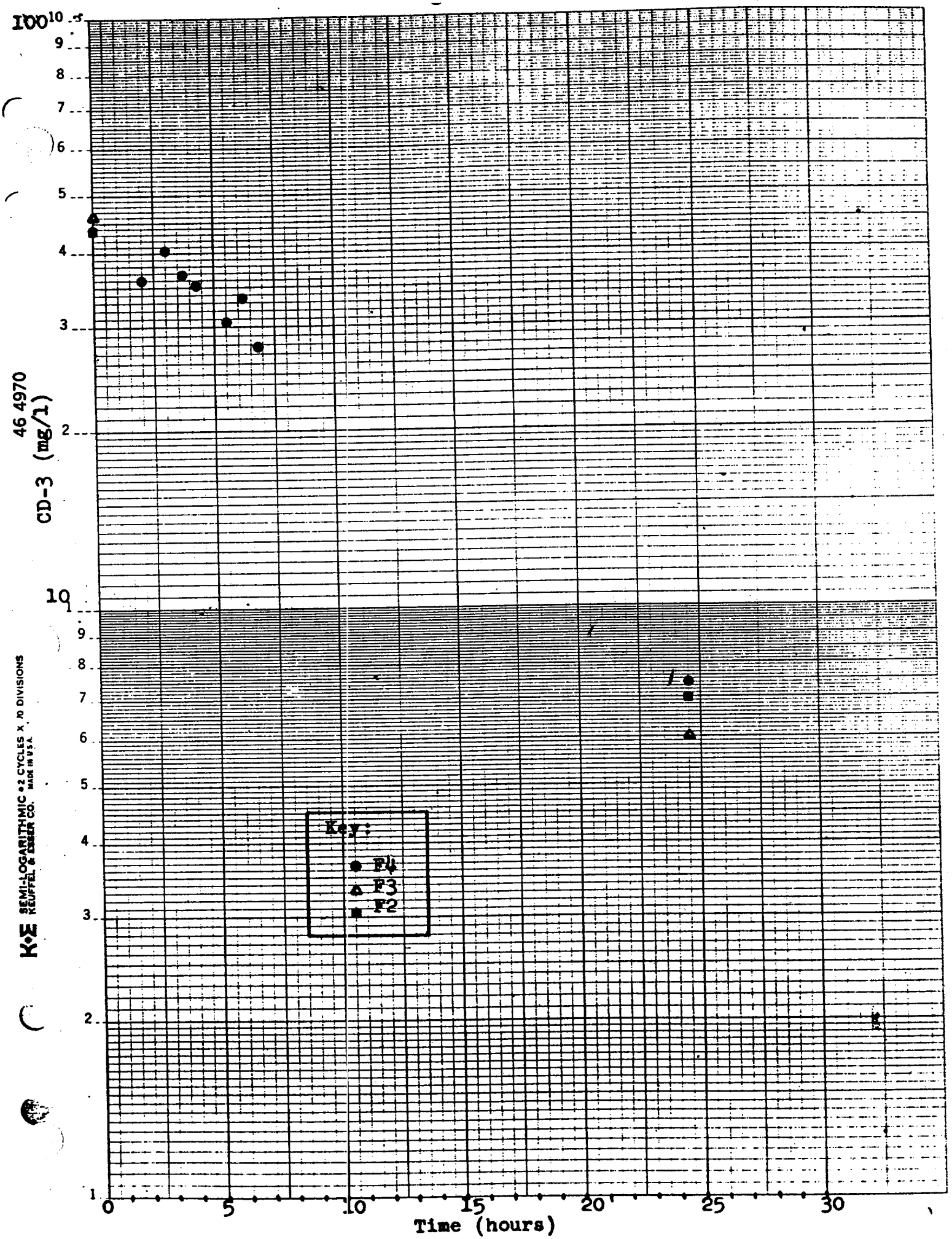

100
10
9
8
7
6
5
4
3
2
1
CD-3 (mg/l)
46 4970
K+E SEMI-LOGARITHMIC • 2 CYCLES X 70 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.
Key:
F4
F3
F2
0
5
10
15
20
25
30
Time (hours)

Table III

Tannin-Lignin

	C4 (mg/l)	F4 (mg/l)	X4 (mg/l)
4/18 0 hrs.	0	12.3	14.2
6 hrs.	0	10.6	11.2
4/19	0	11.7	11.2
4/20	0	9.5	9.0
4/21	0	8.4	7.8

→ PT 905401
904984
903478

CQC/8/81

CATCHMENT QUALITY CONTROL

Report No. 8

The Probable Fate of Certain Phenylenediamines in the Aquatic Environment

CONFIDENTIAL

by

M.L. Richardson

August 1981

Thames Water
Directorate of Scientific Services

Catchment Quality Control,
Mogden Sewage Treatment Works, PO Box 7,
Mogden Lane, Isleworth, Middlesex, England
01-568 5259

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SUMMARY

Phenylene diamine derivatives are widely used as photographic developers and in hair dye preparations.

These compounds were initially thought, through the Catchment Quality Control studies, to be compounds likely to be persistent in the aquatic environment. They were of particular concern in that the predicted river concentrations are large - *circa* 100μg/ℓ.

They are likely to be eliminated either by degradation processes after deamination via hydroquinone or, by polymerisation to Bandrowski's base or humic acid like the compounds which would be adsorbed on sludge.

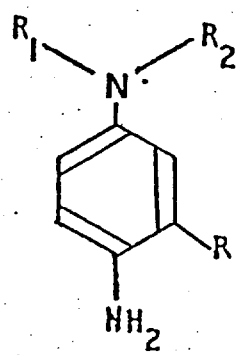
From analytical chemical and biodegradation studies in conjunction with considerations of the biochemistry, it was deduced that they were likely to be so eliminated and hence unlikely to enter potable water supplies.

INTRODUCTION

Certain phenylenediamines are widely used in the photographic industry as colour developing agents and in cosmetic preparations as oxidation dyes.

Catchment Quality Control studies undertaken by Authority (1-4), have indicated the discharge of these compounds to sewage treatment works whose effluents flow into rivers used for potable supply purposes.

Usage within the photographic industry indicates that the quantity discharged is several metric tonnes per annum and is subject to seasonal variation. The cosmetic industry selling preparations for the domestic and professional market uses a similar quantity. These discharges give rise to a calculated concentrations in the Rivers Lee and Thames, or their tributaries, in excess of 100µg/ℓ, assuming no degradation. The compounds generally in use in the photographic industry are p-phenylenediamines (I) having the structure: -



$R = -H$ OR $-CH_3$

$R_1 = -C_2H_5$, OR $-C_2H_4OH$ (I)

$R_2 = -C_2H_5$, $-C_2H_4OH$, OR $-C_2H_4NHSO_2CH_3$

II = 4-amino-N-ethyl-N-β-methanesulfonamidoethyl-m-toluidine sesqui sulfate monohydrate

III = 4-amino-3-methyl-N-ethyl-N-(β-hydroxyethyl) aniline sulfate

IV = p-benzoquinone

METHODS

Analysis of effluents, river water samples etc.

The method of analysis was that recommended by the American National Standards on Photographic Processing Effluents (5) and is based on a coupling reaction with an amine group and a colour coupler; the limit of detection is 10μg/ℓ. It is noted that this coupler could react with other aromatic amines e.g. aniline; such amines are unlikely to be present in the trade effluents concerned.

Aeration experiments

Separate solutions of 1000mg/ℓ of both 4-amino-N-ethyl-N-β-methanesulfonamidoethyl-m-toluidine sesqui sulfate monohydrate (II) and 4-amino-3-methyl-N-ethyl-N-(β-hydroxyethyl) aniline sulfate (III) (Kodak Limited, Harrow, England) and a saturated solution of p-benzoquinone (IV)§ were aerated for 2h and then allowed to stand overnight. In other experiments aeration was continued for four days. The aeration experiments were carried out by simply passing air through a glass tube terminating with a sintered glass disc contained in a 150ml Dreschel bottle at a flow rate of about 150ℓ/hr. The experiments were undertaken in the dark.

Biodegradation tests

These were carried out using OECD and other official procedures (6-12) at the Water Research Centre, Stevenage, in order to ascertain the degree of biodegradation under laboratory conditions.

§ (IV) is considered to be a likely metabolite of (I) in a sewage treatment works environment.

RESULTS

Analysis of effluents etc.

Table 1 indicates the concentrations of p-phenylenediamines found in the trade effluent discharge from a photographic processor (PTP Ltd., Stevenage), the sewage downstream from this discharge, the crude sewage entering the receiving sewage works (Rye Meads), the final effluent from the sewage works and the receiving river.

TABLE 1

Results of Analysis of p-phenylenedimaine derivatives

Samples	p-phenylenediamine concentrations found (µg/ℓ)	p-phenylenediamine concentration predicted (µg/ℓ)
Trade effluent (photographic processor) (Samples taken on three different days)	1390+ 7650+ 195000*	71,000 (from annual discharge quantities)
Crude Sewage 1.3km from trade effluent discharge	33	730
Crude Sewage entering sewage works (Rye Meads)	30	240
Humus Tank Effluent	10	240
River Water (below sewage treatment works (R. Lee))	<10	100
R. Thames - Walton	n.d.†	48
R. Colne - Denham	n.d.†	610

+ Low processing conditions

* Normal processing conditions

† n.d. not determined

Analysis of trade effluent and sewage samples by the Lea Division (13), suggests that the phenylenediamine concentrations tend to decrease with time, distance and dilution, indicating that chemical, biological or physical (adsorption) processes are taking place.

Aeration experiments

Filtered aerated samples (2 days) of (II) and (III) contained no detectable¶ phenylenediamine derivatives when assessed by the coupling method (5). Similarly no p-benzoquinone (IV) was detected on filtered aerated samples by reaction with 2:4-dinitrophenylhydrazine (13), or thio-Michler's ketone (14). Aerated samples of (IV), however, were observed to turn brown (6).

¶ limit of detection 10µg/ℓ

However, samples of all three compounds (II, III and IV) aerated for four days produced a number of unidentified spots when subjected to thin layer chromatography (n-butanol:n-propanol:2N ammonia solution::20:120:60); the spots were considered to indicate the presence of other substances, possibly polymeric.

Biodegradation studies*

The modified OECD screening test (8,9), indicated that neither of the 3-amino-*m*-toluidine derivatives (II and III) was readily biodegradable, within the meaning of 'readily'given to it by the OECD report (9). Even in the presence of a much higher concentration of unacclimatised and acclimatised inocula (20mg activated sludge/ℓ instead of only 0.5mℓ filtered effluent/ℓ, or about a 1000-fold increase, only small proportions (14-35%) of organic carbon were removed on some occasions. In stock solutions both compounds (II and III) were highly coloured and, on standing, the colours deepened, indicating chemical changes. These colours were imparted to the sludges in both the semi-continuous activated sludge (10), and simulation procedures (11). Effluents from the latter were coloured (purple), inferring that some polymerisation may be occurring.

Both compounds, when added to 20mg/ℓ sludge, led to normal growth of sludge in the Husmann test (12), and did not inhibit nitrification, as judged by the almost complete removal of ammonia in all cases. The colour of the effluents interfered with the determination of oxidised nitrogen (reduction to nitrite followed by diazotisation and coupling).

In the simulation test (11), there were large discrepancies between the nominal concentrations of the test substances added to the synthetic sewage, expressed as organic carbon or chemical oxygen demand, and the values obtained by differences. Recoveries ranged from 44% to 75% for organic carbon and from 25% to 56% for chemical oxygen demand. The reasons for these were the limitations of the analytical procedures (relatively small differences between two dissolved organic carbon values) and also the possible chemical changes in the photographic chemicals on standing (q.v. above and below). This unsatisfactory situation - low recoveries of added substances and variable amounts of organic carbon and chemical oxygen demand - was also found in earlier work with surfactants (7), which were known to be readily biodegradable (95%) when assessed by a specific analytical method. Hence, biodegradation may be inferred.

A specific colorimetric method (13), was investigated for the two compounds. However, emulsification at the extraction stage of the determination (6) caused the loss of varying amounts of the complex dye formed in the reactions. This led to low variable values when the method was applied to synthetic sewage (another possible reason for the low recovery is the auto-oxidation which these chemicals undergo in solution).

* All these studies were carried out in the laboratories of the Water Research Centre (Stevenage).

Considering the biodegradation of p-benzoquinone, (IV), Painter and King (6) reported that evidence of U.V. absorption and dissolved organic carbon removals clearly show that p-benzoquinone is very readily ultimately biodegraded. However, significant oxygen uptake was recorded only in the presence of p-benzoquinone acclimated sludge, and the compound was mildly inhibitory to sewage sludge mixture (26% at 20mg carbon/ℓ). It is appreciated that these biodegradation tests were undertaken in the mg/ℓ range, whereas in the systems under consideration the compounds would be present only in the μg/ℓ range, where co-metabolism could play a vital role. A biodegradation study in the μg/ℓ range would, therefore, assist in ascertaining the fate of these compounds in the aquatic environment.

DISCUSSION

In view of the above results of analysis and biodegradation experiments, indicating that p-phenylenediamine derivatives (I) and their probable metabolite(s)(IV), disappear in the aquatic environment, proposed biological and chemical pathways were considered and these pathways are discussed below.

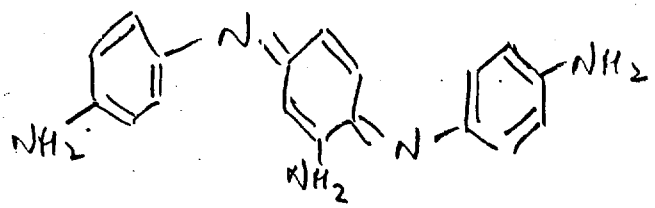
Martin (16) suggests that p-phenylenediamines can be enzymatically oxidised to p-benzoquinone at low concentrations at approximately pH 7, i.e. the case in the aquatic environment. If benzoquinones (IV) are formed from colour developers, (no analytical evidence has been obtained for their formation in sewage works effluent or rivers) and as they are chemically active compounds, then they are likely to be subject to further reactions.

In neutral or basic solutions hydroquinone (formed by the hydrolysis of benzoquinone) and the benzoquinone are unstable (17) and likely to lead by polymerisation to humic acid type substances. These are probably adsorbed on sludge, making detection impossible, but may be one of the unidentified TLC spots previously referred to.

During hydrolysis, hydroxylation in the 2-,3-, and 4- positions on the benzene ring can occur (17). Should this occur, ring scission by the well known ortho and meta pathways (18) will occur to yield muconic acid which will, in turn, degrade ultimately to carbon dioxide and water.

Additionally Erdmann (19) and Wilstätter and Meyer (20), indicate that the first stage of oxidation of p-phenylenediamine is the formation of the quinonedi-imine (21). Further reaction between the di-imine and diamine gives the dark garnet coloured product Bandrowski's base (22,23,24) which is the basis of many hair dyes. Further reactions (25) would lead to dyestuffs which would be readily adsorbed onto sewage sludge, again making detection impossible.

Analysis has indicated that the levels of p-phenylenediamine derivatives in the River Lee, which can contain much sewage works effluent, are unlikely to exceed 10µg/ℓ. In order to clarify the foregoing, especially as the mg/ℓ biodegradation tests are equivocable, it is recommended that a ring labelled ^{14}Cµg/ℓ biodegradation study is undertaken.



EPIDEMIOLOGY

Hydroquinone (oxidisable to p-benzoquinone has been used as a photographic developer for over 100 years and p-phenylene-diamine derivatives have been used commercially as colour developers since 1935. Over these years no epidemiological study, known to the photographic industry, has ever shown that enthusiastic amateurs or professionals involved in processing photographic material are subject to any special mutagenic or carcinogenic risk(17). The time spanned covers the era when fingers, hand and sometimes even whole arms were dipped into processing solutions without protective wear.

The International Agency for Research on Cancer (26) has stated that the epidemiological evidence currently available suggests an elevated risk for both users of hair dyes and those (barbers and hairdressers) occupationally exposed to hair preparations (27). It is further suggested (26) that epidemiological studies, which should include workers employed in the production of hair dyes, are required before firm conclusions are drawn.

CONCLUSIONS

From the foregoing it can be deduced that p-phenylenediamines, as used in both hair dyeing preparations and as photographic colour developers, are likely either to degrade ultimately or react/polymerise and therefore, in spite of lack of evidence, be removed from the aquatic environment, on sewage works sludge or river benthic deposits.

Hence, there is unlikely to be any long-term health hazard from the constant ingestion of potable water abstracted from rivers containing sewage works effluent which, in turn, contains trade or domestic effluents containing p-phenylenediamines.

There is no epidemiological evidence from the photographic industry that indicates a long-term health risk from using these compounds. However, the continued use of hair-dyes by persons in the home and by professionals in the hairdressing trade may lead to an elevated risk.

Finally, in order to clarify the fate of these compounds it was concluded that a ^{14}Cµg/ℓ biodegradation study should be undertaken. This is of importance in view of the significant quantities of the compounds discharged.

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7. Water Research Centre - Dr. H.A. Painter and Miss E. King (for the biodegradation studies).

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FINAL REPORT

"EASTMAN" COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 EAN: 905401
CAS No.: 025646-71-3

SKIN SENSITIZATION STUDY IN THE GUINEA PIG

AUTHOR

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STUDY SPONSOR

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REPORT DATE

August 7, 1997

"EASTMAN" COLOR DEVELOPING AGENT CD-3

PM No.: 01578-00 EAN: 905401
CAS No.: 025646-71-3

SKIN SENSITIZATION STUDY IN THE GUINEA PIG

A dermal sensitization study was conducted with this test substance in guinea pigs using a standardized method developed by the Laboratory of Industrial Medicine, Eastman Kodak Company. The study was initiated on January 24, 1956. All data from this study were stored in the testing facility archive managed under GLP-mandated conditions. This report was prepared from the archived raw data.

For this study, twenty guinea pigs were used. On the first day of the study (Day 0), individual body weights were recorded and the backs of the guinea pigs were clipped and depilated. A solvent-vehicle was prepared using 4.4 mL of chloroform added to 5.4 mL of a solution of acetone, dioxane, and guinea pig fat (7:2:1). Ten of the twenty guinea pigs were assigned to a test group which was exposed to the test substance in the solvent-vehicle (0.1M). Five of the remaining ten guinea pigs were assigned to a positive control group and exposed to phenylhydrazine hydrochloride, a known dermal sensitizer, in the solvent-vehicle (0.1M). The last five guinea pigs were assigned to a negative control group and exposed only to the solvent-vehicle.

The induction phase of the study consisted of topical exposures to the appropriate material (test substance, positive control substance, or solvent-vehicle) in a volume of 7 drops on Days 0, 2, and 4. At 24 and 48 hours after the first exposure on Day 0, initial readings of redness (R) and swelling (S) were made at each of the application sites. Irritation responses were graded using the appended 4-point scale described by Draize et al. (J. Pharmacol. Exp. Therap. 82:377, 1944). No exposures were performed during the subsequent three weeks. After the three-week rest period, the three groups of animals received two additional exposures (challenge and rechallenge) one week apart (Days 34 and 41) using a volume of 10 drops of the appropriate material for each exposure. The application sites were scored 24 and 48 hours after each application.

Sensitization by the test substance was evaluated by comparing the final set of readings with the readings made after the first induction exposure. Results obtained after treatment of the test substance, positive control substance, and the solvent-vehicle are presented in Tables 1 - 3.

TABLE 1

0.1 M "EASTMAN" Color Developing Agent CD-3 in Solvent-Vehicle

Animal Number	Initial Weight (g)	Dermal Scores (R,S) R = Redness S = Swelling					
	01/24/56	01/25/56	01/26/56	02/21/56	02/22/56	02/28/56	02/29/56
765	566	1,0	1,0	1,0	1,0	1,0	1,0
766	549	1,0	1,0	1,0	0,0	1,0	0,0
767	521	1,0	0,0	1,0	1,0	1,0	1,0
768	434	1,0	1,0	1,0	2,0	1,0	1,0
769	513	1,0	1,0	2,0	2,0	1,0	2,0
770	587	1,0	1,0	1,0	1,0	1,0	2,0
771	476	1,0	1,0	3,0	2,0	2,0	1,0
772	504	1,0	1,0	2,0	1,0	1,0	1,0
773	505	0,0	1,0	1,0	1,0	1,0	1,0
774	478	0,0	1,0	2,0	1,0	2,0	1,0

TABLE 2

0.1 M Phenylhydrazine hydrochloride (Positive Control) in Solvent-Vehicle

Animal Number	Initial Weight (g)	Dermal Scores (R,S) R = Redness S = Swelling					
	01/24/56	01/25/56	01/26/56	02/21/56	02/22/56	02/28/56	02/29/56
795	390	1,0	3,0	3,0	3,0	3,0	3,0
796	451	1,0	3,0	3,0	4,1	3,0	3,0
797	457	1,0	3,0	3,0	3,1	3,0	3,0
798	363	1,0	3,0	3,0	3,0	3,0	3,0
799	457	2,0	3,0	2,0	3,0	3,0	3,0

TABLE 3

Solvent-Vehicle (Negative Control)

Animal Number	Initial Weight (g)	Dermal Scores (R,S) R = Redness S = Swelling					
	01/24/56	01/25/56	01/26/56	02/21/56	02/22/56	02/28/56	02/29/56
800	372	1,0	1,0	1,0	1,0	1,0	1,0
801	344	1,0	1,0	0,0	1,0	1,0	0,0
802	404	1,0	1,0	1,0	1,0	1,0	1,0
803	421	1,0	1,0	1,0	0,0	0,0	0,0
804	394	1,0	1,0	1,0	1,0	1,0	1,0

DISCUSSION

Under the conditions of this study, there was evidence of sensitization by the test substance. For the ten animals induced with the test substance, scores for redness and swelling at challenge were higher for than those scores obtained after the first induction. In addition, scores at challenge for the five animals induced with the positive control substance were also higher than the initial scores at induction. In contrast, the negative control solvent-vehicle produced no evidence of dermal sensitization. Although the scores at challenge for the test substance were not as severe as the scores produced by the positive control substance, they did indicate a moderate dermal reaction caused by the test substance.

CONCLUSION

Based on these observations, the test substance was considered to be a dermal sensitizer in guinea pigs and has a moderate potential for human dermal sensitization.

APPENDIX

The most severely affected area within in the site of application of the test substance was scored for erythema and edema. The following scale, published by Draize, et al. (1944) was used for scoring.

GRADING OF SKIN REACTION

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema to slight eschar formation	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (edges raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm and extending beyond area of exposure)	4

MW = 33.03 MW = 129.10

PLEASE RETURN TO DR. ROUDABUSH

9541

TOXICITY REPORT - E.K.CO. - LABORATORY OF INDUSTRIAL MEDICINE

Chemical: D_2W Developer, with and without Hydroxylamine

No:

Source: C. Mens Color Proc. Dev.

Formula: H, H, R-OH as sulfate $(NH_2OH)_2SO_4$

Solution	Animals* No. and Species	Route**	Dose Range	Approx. LD_{50}	Symptoms	Time of Death	Wt. Change 2 wks
Acute Toxicity			mg/kg	mg/kg	Notebook No.	P	
Skin Absorption and Irritation			cc/kg	cc/kg			
D_2W Developer + Hydroxylamine	3 G.P.	Rubber cuff & gauze pad	5 - 20	> 20	No or slight edema, moderate redness, brown stain	—	1-, 2+
D_2W Developer (control)	3 G.P.	"	5 - 20	> 20	No edema, very slight redness	—	2+, 1-
					Notebook No.	A 60 P	44

*G.P. - Guinea Pig, M - Mouse, R - Rat, RB - Rabbit

**PO - Orally, IP - Intraperintoneally, IM - Intramuscularly, IC - Intracutaneously

6/9/54

Remarks: D_2W with hydroxylamine is more irritating than D_2W alone.

KP 42868

905401

Exp. No. _____

SENSITIZATION

Compound CD 3 [illegible] HSL No. 73-[illegible]

Source _____ Date [illegible]

Guinea pig: Male Female Solution [illegible]

Type test: Drop on Footpad inj.-drop on Cuff-rub on-cuff

Animal Number	Weight		Erythema and Swelling								
	6-13-73	7-25-73	6-14	6-15	7-[illegible]	7-12	7-17	7-18	7-24	7-25	
997	395	600	10	10	10	10	10	20	20	20	
998	[illegible]	[illegible]	20	20	[illegible]	20	[illegible]	[illegible]	[illegible]	[illegible]	
999	470	700	10	10	00	10	10	10	10	10	
1000	385	525	10	10	10	20	10	10	20	10	
1	425	700	20	20	30	30	30	30	30	30	
2	351	610	10	20	10	10	10	10	10	10	
3	385	620	20	20	20	20	20	10	10	10	
4	380	590	10	20	20	20	20	20	20	30	
[illegible]	[illegible]	[illegible]	10	10		20	20	[illegible]	20	[illegible]	
6	[illegible]	[illegible]	20	20	[illegible]	20	[illegible]	[illegible]	20	20	

Control Summary

Select Control [illegible]	1.2	1.0	0.7	0.9	1.0	1.0	1.1	0.7
[illegible]	1.8	[illegible]	[illegible]	[illegible]	5.6	5.3	[illegible]	5.4

Acc. No. 905401 06 TOXICITY REPORT - EASTMAN KODAK COMPANY - HEALTH AND SAFETY LABORATORY HSL No. 78-506 905401

Chemical: CD-3 Source: C. J. Terhaar Formula:

Solution	Animals* No. and Species	Type of Test	Initial Score		Final Score		
			24 hrs	48 hrs	24 hrs	48 hrs	
Skin Sensitization							
1% in 80% H_2O + 20% A, D and G.P. fat	5 G.P.	Drop on	1.2	1.2			
Footpad inj.-1% in Freund's adj.; drop on-1% in 80% H_2O + 20% A, D and G.P. fat	5 G.P.	Footpad-drop on			1.8	2.2	
Footpad inj.-plain Freund's adj.; drop on-1% in 80% H_2O + 20% A, D and G.P. fat	5 G.P.	Footpad-drop on			1.0	1.2	

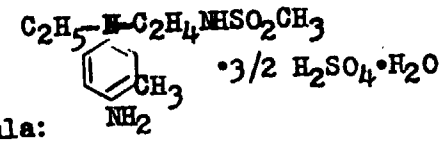
*G.P. = Guinea Pig

2-12-79/bdo

Remarks: Sensitized 1/5 guinea pigs. Moderate activity.

~~825[illegible]~~ 905401 06

TOXICITY REPORT - E.K.CO. - LABORATORY OF INDUSTRIAL MEDICINE

Formula: 

Chemical: D_2W No: Source: Dr. Weissberger

Solution	Animals* No. and Species	Route**	Dose Range	Approx. LD_{50}	Symptoms	Time of Death	Wt. Change 2 wks
Acute Toxicity			mg/kg	mg/kg			
10% in water	5 R	P.O.	50 - 800	400-800	Weakness, ataxia.	15 Min	4 ↓
10% in water	7 R	I.P.	10 - 400	< 10	Weakness, ataxia, unconsciousness, gasping, mild convulsion in highest dose.	14 Min-11 days	1 ↓ *
					Notebook No.	▲ 72 P	33
Skin Absorption and Irritation			cc/kg	cc/kg			
					Notebook No.	P	

* 2 Rats were done on 25 mg/kg. One survived and gained weight and the other died in 11 days. However rat receiving 10 mg/kg died in 6 days.

4/21/55

*G.P. - Guinea Pig, M - Mouse, R - Rat, RB - Rabbit

**PO - Orally, IP - Intraperintoneally, IM - Intramuscularly, IC - Intracutaneously

Remarks: Slightly toxic orally in rats, highly toxic intraperitoneally.

KP 42868

Acc. No. 905401

Vascular Phytotoxicity Report—E.K. Co.—Health and Safety Laboratory

HSL 74-243

Chemical: CD-3

Source: Photo Tech

No. and Type Plant	Test*	Date	Dose Range mg or µl/l	Germinated No.	Germinated %	Av. Hypocotyl Length (cm)	Av. Root Length (cm)	Hyp./Root Ratio	Plant Growth (cm)	Conditions (wks)
10 Corn	P	2-18-75	1000 mg/1**				30.3		19.2	Few leaves with light brown blotches, roots dark tan color.
10 Corn	P	"	Control**				28.3		20.5	Normal.
10 Corn	P	10-14-75	100 mg/1**				32.8		17.6	Normal.
10 Corn	P	"	Control**				35.6		19.1	Normal.
20 Marigold	P	2-18-75	1000 mg/1**				19.6		2.5	Some leaves with light brown specks on margins, roots, sl. inhibited.
20 Marigold	P	"	Control**				21.3		2.7	Normal.
20 Marigold	P	10-14-75	100 mg/1**				15.8		2.0	Normal.
20 Marigold	P	"	Control**				12.3		1.8	Roots sl. short.
20 Lettuce	P	2-18-75	1000 mg/1**				8.5		2.6	Leaves with sl. brown splotches, several dried. Plant growth sl. inhibited.
20 Lettuce	P	"	Control**				8.7		3.6	Petioles sl. weak.
20 Lettuce	P	10-14-75	100 mg/1**				8.0		4.3	Normal.
20 Lettuce	P	"	Control**				7.2		2.2	Normal.
20 Radish	P	2-18-75	1000 mg/1**				17.1		2.4	Leaves with brown splotches, several dried, some shriveled. Sl. root and growth inhibit.
20 Radish	P	"	Control**				18.1		2.8	Normal.
20 Radish	P	10-14-75	100 mg/1**				16.1		5.6	Normal.
20 Radish	P	"	Control**				15.3		4.4	Normal.

*B = 3-month Bioaccumulation Test—greenhouse; G = 7-day Germination Test—etiolated; P = 1-week Phytotoxicity Test conducted on 2-week-old vascular plants—greenhouse; ** = Photograph taken

12-17-75/bdo

Remarks:

KP6 69076A

Chemical: Kodak Color Developing Agent Type CD_3 No: Source: E. R. Taylor Mfg. Expts. Formula:

Solution	Animals* No. and Species	Route**	Dose Range	Approx. LD_{50}	Symptoms	Time of Death	Wt. Change 2 wks
Acute Toxicity			mg/kg	mg/kg			
20% in water	9 R	P.O.	10 - 3200	100-200	Weakness, ataxia, mild hopping convulsions, unconsciousness.	15M-1 day	4 ↓
					Notebook No.	A 76 p	33
Skin Absorption and Irritation			cc/kg	cc/kg			
					Notebook No.	P	

*G.P. - Guinea Pig, M - Mouse, R - Rat, RB - Rabbit

**PO - Orally, IP - Intraperintoneally, IM - Intramuscularly, IC - Intracutaneously

11/4/55

Remarks: Moderately toxic compound.

Acc. No. 905401

Vascular Phytotoxicity Report—E.K. Co.—Health and Safety Laboratory

HSL 74-243

905401

Chemical: CD-3

Source: Photo Tech

No. and Type Plant	Test*	Date	Dose Range mg or µl/l	Germinated No.	Germinated %	Av. Hypocotyl Length (cm)	Av. Root Length (cm)	Hyp./Root Ratio	Plant Growth (cm)	Conditions (wks)
		1975								
80 Ryegrass	G	7-31	1000 mg/1**	73	91	4.6	1.1	4.2		Roots inhibited, irratic lengths or lacking (10%).
80 Ryegrass	G	7-31	100 mg/1	67	84	4.8	2.8	1.7		Normal.
40 Ryegrass	G	7-31	Control	37	93	4.5	3.0	1.5		Normal.
80 Radish	G	7-31	1000 mg/1**	78	98	7.3	8.5	0.9		Hypocotyl wk. and inhibited, roots and hair roots inhibited.
80 Radish	G	7-31	100 mg/1	79	99	8.0	13.5	0.6		Some roots inhibited, inhibited hair roots and yellow tips.
80 Radish	G	7-31	10 mg/1	80	100	8.0	15.8	0.5		Normal.
40 Radish	G	7-31	Control	40	100	8.5	13.5	0.6		Normal.
80 Lettuce	G	7-31	1000 mg/1**	80	100	3.0	1.4	2.1		Hypocotyls and roots inhibited and irratic lengths. Some cotyledons were black.
80 Lettuce	G	7-31	100 mg/1	77	96	3.8	3.4	1.1		Sl. root inhibition.
80 Lettuce	G	7-31	10 mg/1	78	98	3.9	3.8	1.0		Normal.
40 Lettuce	G	7-31	Control	40	100	4.0	3.8	1.1		Normal.

*B = 3-month Bioaccumulation Test—greenhouse; G = 7-day Germination Test—etiolated; P = 1-week Phytotoxicity Test conducted on 2-week-old vascular plants—greenhouse; ** = Photograph taken

10-12-76/bdo

Remarks:

KP 69076A