

**STUDY TITLE**

**ACUTE ORAL TOXICITY STUDY IN THE RAT**

**FINAL REPORT**

**AUTHOR**

**Kenneth P. Shepard, B.S.**

**PERFORMING LABORATORY**

**Toxicological Sciences Laboratory  
Health and Environment Laboratories  
Eastman Kodak Company  
1100 Ridgeway Avenue  
B-320 Kodak Park  
Rochester, New York 14652-6272  
USA**

**LABORATORY PROJECT ID**

**HAEL Number: 94-0031**

**STUDY SPONSOR**

**Eastman Kodak Company**

**STUDY COMPLETION DATE**

**April 12, 1995**

**QUALITY ASSURANCE INSPECTION STATEMENT**

[21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), and 40 CFR 160.35(B)(7)]

STUDY: STUDY DIRECTOR: SHEPARD, K.P. PAGE 1  
 ACCESSION NUMBER: 03/31/95

STUDY TYPE: ACUTE ORAL TOXICITY

*M. S. James*  
 (AUDITOR, QUALITY ASSURANCE UNIT)

*April 10, 1995*  
 DATE

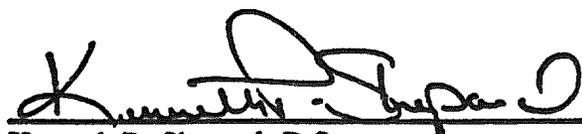
-----  
 TO THE BEST OF MY KNOWLEDGE, THIS FINAL REPORT ACCURATELY DESCRIBES THE METHODS AND STANDARD OPERATING PROCEDURES, AND THE REPORTED RESULTS ACCURATELY REFLECT THE RAW DATA. THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT OF HAEL, EASTMAN KODAK COMPANY ROCHESTER, N.Y. AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:  
 -----

<u>INSPECTION DATES</u>	<u>PHASE(S) INSPECTED</u>	<u>STATUS REPORT DATES</u>
05/02/94	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
05/05/94	CLINICAL SIGNS AT 72 HRS.	
07/19/94	PROTOCOL APPENDIX/AMENDMENT SUBMISSION REPEAT - LOWER DOSES	
07/21/94	CLINICAL SIGNS AT 48 HRS.	
11/04/94	GROSS PATHOLOGY HISTOPATHOLOGY PATHOLOGY REPORT	11/04/94
03/31/95	FINAL REPORT REVIEW	03/31/95

**COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS**

The study described by this report was conducted in compliance with the following Good Laboratory Practice Standards:

Annex 2 of the Organization for Economic Cooperation and Development Guidelines for Testing of Chemicals C(81)30 (Final).

  
\_\_\_\_\_  
Kenneth P. Shepard, B.S.  
Study Director

4/12/95  
\_\_\_\_\_  
Month/Day/Year

<u>TABLE OF CONTENTS</u>	Page Number
ABSTRACT	5
PERFORMING LABORATORY	7
SPONSOR	7
STUDY DATES	7
STUDY DIRECTOR	7
OTHER KEY PERSONNEL	7
PURPOSE/OBJECTIVE	8
TEST SUBSTANCE	8
TEST SYSTEM	8
HUSBANDRY AND ENVIRONMENTAL CONDITIONS	8
TEST PROCEDURES AND CONDITIONS	9
RESULTS	11
DATA ANALYSIS	25
DISCUSSION AND INTERPRETATION	25
CONCLUSION	26
DATA STORAGE	26
REFERENCES	26
SIGNATURE PAGE	27
PATHOLOGIST'S REPORT	Appended

**ABSTRACT**

**ACUTE ORAL TOXICITY STUDY IN THE RAT**

**HAEL NUMBER:                      KAN:  
CIN:**

An acute oral toxicity study was initially conducted in five male and five female rats administered a single limit dose of 2000 mg/kg of the test material by gavage. Within 24 hours of dosing, four animals of each sex died. The remaining male and female at this dose level died on Day 6 and Day 3 of the study, respectively. Based on results of two range-finding tests, three additional groups of five males and five females were administered 125, 250 or 500 mg/kg of the test material. In the oral toxicity study, mortality noted at the 500 mg/kg dose level included a male on Day 10, single females on Days 1 and 3, and two females on Day 12 of the study. At the 250 mg/kg dose level, a male died on Day 2 and single females died on Day 3 and Day 7. At the 125 mg/kg dose level, mortality was limited to a single female on Day 3 of the study. No other mortality was noted during the study.

Abnormal clinical signs evident during the 14-day observation period included slight to severe weakness, a reduced amount or lack of feces, discolored (brown) urine, staining (yellow) of the inguinal hair, dehydration, porphyrin staining of the facial hair, and rough haircoat. Alopecia of the abdomen was observed for a single female at the 500 mg/kg dose level. The test material, a green solid, also caused blue diarrhea and blue staining or discoloration of the feces; skin of the tail; and hair of the abdomen, face and inguinal area. A body weight loss was recorded for all eight surviving animals at the 500 mg/kg dose level and for three of seven surviving animals at the 250 mg/kg dose level during the first week of the study. During the second week of the study, a body weight loss was also recorded for a single animal of each sex at the 500 mg/kg dose level. All animals in the 125 mg/kg dose group which survived gained weight during both weeks of the study.

The cause of death for rats which died after exposure to the test material was not determined. The major treatment-related changes noted at necropsy included test material in the trachea and gastrointestinal tract; blue discoloration by the test material observed in the mucosa of the trachea and gastrointestinal tract; blue discoloration of the skin of the feet and tail, and the inguinal and facial hair; hemorrhage in the lungs; and nasal porphyrin discharge. The remaining treatment-related changes were considered to be secondary to loss of body weight and/or stress-associated sporadic lesions which were observed in a few rats.

**ABSTRACT. Cont.**

The acute oral LD<sub>50</sub> for this test material was calculated to be 794 mg/kg for male rats and 315 mg/kg for female rats. The acute oral LD<sub>50</sub> calculated by combining the male and female mortality data was 583 mg/kg. Based on the oral LD<sub>50</sub> calculated from the combined mortality data, the test material was classified as slightly toxic in rats according to the criteria set forth by Hodge and Sterner (1949) and classified as harmful if swallowed as defined in the 18th Adaptation on the EC Classification, Packaging, and Labelling of Dangerous Substances.

**PERFORMING LABORATORY**

Toxicological Sciences Laboratory  
Health and Environment Laboratories  
Eastman Kodak Company  
1100 Ridgeway Avenue  
B-320 Kodak Park  
Rochester, New York 14652-6272  
USA

**SPONSOR**

Eastman Kodak Company

**STUDY DATES**

Study Initiation: May 2, 1994  
Experiment Initiation: May 2, 1994  
Experiment Completion: December 14, 1994  
Study Completion: April 12, 1995

**STUDY DIRECTOR**

Kenneth P. Shepard, B.S.

**OTHER KEY PERSONNEL**

Leonard Sakal, B.S., Study Technician  
John W. Mosher, B.S., Principal Investigator  
Milan S. Vlaovic, D.V.M., Ph.D., Pathologist, Laboratory Animal Medicine

**PURPOSE/OBJECTIVE**

The purpose of the study was to determine the estimated oral LD<sub>50</sub> of the test material in male and female rats and the clinical signs of toxicity associated with a single oral dose.

**TEST SUBSTANCE**

Physical State and Appearance: Green solid  
Received at Performing Laboratory: April 28, 1994  
Composition: Refer to composition information included in the notification when applicable.

**TEST SYSTEM**

Species:	Rat
Strain:	CD®(SD)BR VAF/Plus®
Source:	Charles River Laboratories, Kingston, NY, USA
Sex:	Male and Female
Number of Animals:	Five of each sex per dose group
Body Weight Range (grams):	Males = 177 - 236 Females = 155 - 203
Age at Study Initiation:	Males = 6-7 weeks Females = 7-8 weeks

**HUSBANDRY AND ENVIRONMENTAL CONDITIONS**

**Environmental Conditions**

A photoperiod of 12 hours light from 6 a.m. to 6 p.m. was maintained. Room temperature was maintained at 67-72 °F. With the exception of a single day when relative humidity transiently reached 73%, relative humidity was maintained at 49-65%.

**HUSBANDRY AND ENVIRONMENTAL CONDITIONS, Cont.**

**Housing**

All animals were individually housed in suspended, stainless-steel, mesh cages.

**Diet and Water**

Agway® ProLab™ Animal Diet - RMH 3000 (certified) pellets and water (Monroe County (NY) Water Authority) were available *ad libitum*. No known contaminants which would interfere with the outcome of the study were expected to be present in feed or water from these sources. Analyses of feed and results of quarterly analyses of water are maintained on file within the testing laboratory.

**Isolation**

Animals were isolated and monitored for at least five days after arrival and before release to the testing facility.

**Animal Identification**

All animals were identified by cage numbers and uniquely-numbered metal ear tags.

**TEST PROCEDURES AND CONDITIONS**

**Test Procedure Guideline**

OECD Guideline for Testing of Chemicals: Guideline 401 (Annex V, test B.1).

**Randomization**

A clinical examination was performed on each animal to ensure that only healthy animals were utilized. 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 using the Automated Animal Toxicology System. After assignment of animals to the study, the body weights were determined to ensure that individual body weights did not exceed 20% of the mean weight for each sex.

**TEST PROCEDURES AND CONDITIONS. Cont.****Identification Numbers of Animals Used**

<u>Range-Finding Tests</u>	<u>Males</u>	<u>Females</u>
250 mg/kg	961	964
500 mg/kg	962	965
1000 mg/kg	963	966
100 mg/kg	81	84
200 mg/kg	82	85
400 mg/kg	83	86

<u>Oral Toxicity Study</u>	<u>Males</u>	<u>Females</u>
125 mg/kg	241-245	256-260
250 mg/kg	246-250	261-265
500 mg/kg	251-255	266-270
2000 mg/kg	911-915	916-920

**Dose Levels**

Initially, a limit dose of 2000 mg/kg was administered to the animals. Based on the mortality rate at this dose level and the results of two range-finding tests, three additional dose levels of 125, 250, and 500 mg/kg were added to the study.

**Dosing Regimen**

The test material was administered as a single dose to animals that had been fasted overnight. Variability in test volumes was minimized by adjusting the concentration to ensure a constant volume at all dose levels. The test material was administered by gavage as a 1.25, 2.5, 5, or 20% suspension in the vehicle.

**Vehicle and Control Substance**

The vehicle was a 0.5% aqueous suspension of guar gum (Jaguar®), Control Number: F-10-88-592-10. No control substance was used.

## TEST PROCEDURES AND CONDITIONS. Cont.

### Clinical Observations

Animals were observed three times on the day of dosing (Day 0), and once each day thereafter for the duration of the experiment (a total of 14 calendar days). Observations included, but were not limited to, changes in the skin; fur; feces; urine; eyes; mucous membranes; respiratory, circulatory, autonomic, and central nervous systems; somatomotor activity; and behavior pattern.

### Body Weight Determinations

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

### Necropsy

Animals that died during the study were necropsied as soon as possible. Surviving animals were necropsied at the completion of the 14-day observation period.

## RESULTS

### Range-Finding Test

After administration of a limit dose of 2000 mg/kg, two range-finding tests were conducted to select dose levels for the oral toxicity study. In the first range-finding test, one animal of each sex was administered a single dose of 250, 500, or 1000 mg/kg. For these three dose groups, the test material was administered as a 10% suspension in a 0.5% aqueous suspension of guar gum. With the exception of the female at the 250 mg/kg dose level, all animals died after administration of the test material. Therefore, a second range-finding test was initiated using one animal of each sex administered doses of 100, 200, or 400 mg/kg of the test material. In the second test, variability in test volumes was minimized by adjusting the concentration to ensure a constant volume at all dose levels. For this test, the material was administered as a 1, 2, or 4% suspension in the same guar gum vehicle used in the first range-finding test. The male at the 400 mg/kg dose level died after dosing. No other deaths occurred in the second range-finding test during a 14-day period. Based on the range-finding tests, dose levels of 125, 250, and 500 mg/kg were selected for the oral toxicity study.

**RESULTS. Cont.****Oral Toxicity Study****Mortality**

In the oral toxicity study, mortality was 0% at 125 mg/kg, 20% at 250 and 500 mg/kg, and 100% at 2000 mg/kg for male rats. For the females, mortality was 20% at 125 mg/kg, 40% at 250 mg/kg, 80% at 500 mg/kg, and 100% at 2000 mg/kg.

**MORTALITY TABLE**

<b>DOSE (mg/kg)</b>	<b>NUMBER OF RATS EXPOSED (Male, Female)</b>	<b>NUMBER OF DEATHS (Male, Female)</b>	<b>TIME OF DEATH</b>
125	5,5	0,1	Day 3
250	5,5	1,2	Day 2 to Day 7
500	5,5	1,4	Day 1 to Day 12
2000	5,5	5,5	Day 1 to Day 6

**Clinical Observations**

Abnormal clinical signs evident during the 14-day observation period included slight to severe weakness, a reduced amount or lack of feces, discolored (brown) urine, staining (yellow) of the inguinal hair, dehydration, porphyrin staining of the facial hair, and rough haircoat. Alopecia of the abdomen was observed for a single female at the 500 mg/kg dose level. The test material, a green solid, also caused blue diarrhea and blue staining or discoloration of the feces; skin of the tail; and hair of the abdomen, face and inguinal area. The time of each observation and the number of animals involved at each dose level are listed in the following table.

**RESULTS. Cont.****TABLE OF CLINICAL OBSERVATIONS**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
125	Day 0 Day 0 Day 0	Normal Blue Diarrhea Staining (Blue) of the Inguinal Hair	1/5 Females 3/5 Males, 4/5 Females 3/5 Males, 3/5 Females
125	Day 1 Day 1 Day 1 Days 1-3 Days 1-4 Days 1-5	Staining (Blue) of the Inguinal Hair Reduced Amount of Feces Staining (Blue) of the Skin of the Tail Discolored (Blue) Feces Discolored (Blue) Feces Staining (Blue) of the Skin of the Tail	4/5 Males, 5/5 Females 5/5 Males 5/5 Females 4/5 Females 5/5 Males 5/5 Females 3/5 Males
125	Day 2 Day 2 Day 2 Day 2 Day 2 Day 2 Day 2	Staining (Blue) of the Inguinal Hair Slight Weakness Moderate Weakness Reduced Amount of Feces Staining (Blue) of the Skin of the Tail Staining (Yellow) of the Inguinal Hair Dehydration Porphyrin Staining of the Facial Hair	3/5 Males, 4/5 Females 1/5 Females 1/5 Females 1/5 Females 3/5 Females 1/5 Females 2/5 Females 1/5 Females
125	Day 3 Day 3 Days 3-4 Days 3-5	Death Staining (Blue) of the Inguinal Hair Staining (Blue) of the Skin of the Tail Staining (Yellow) of the Inguinal Hair	1/5 Females 2/5 Males, 3/4 Females 2/4 Females 1/4 Females
125	Day 4 Days 4-5 Days 4-5	Discolored (Blue) Feces Normal Staining (Blue) of the Inguinal Hair	3/5 Males, 1/4 Females 1/5 Males 1/5 Males, 2/4 Females
125	Day 5 Day 5	Discolored (Blue) Feces Staining (Blue) of the Skin of the Tail	2/5 Males 3/4 Females
125	Day 6 Day 6 Days 6-8 Days 6-7	Normal Staining (Blue) of the Skin of the Tail Staining (Blue) of the Skin of the Tail Staining (Blue) of the Inguinal Hair	3/5 Males, 3/4 Females 2/5 Males 1/4 Females 1/4 Females
125	Days 7-12 Days 7-14 Days 7-14	Normal Normal Staining (Blue) of the Skin of the Tail	3/4 Females 4/5 Males 1/5 Males
125	Days 13-14	Normal	4/4 Females

**RESULTS. Cont.****TABLE OF CLINICAL OBSERVATIONS**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
250	Day 0	Normal	1/5 Males, 1/5 Females
	Day 0	Blue Diarrhea	4/5 Males, 4/5 Females
	Day 0	Staining (Blue) of the Inguinal Hair	4/5 Males, 1/5 Females
250	Day 1	Slight Weakness	2/5 Males
	Day 1	Staining (Blue) of the Inguinal Hair	5/5 Males
	Day 1	Reduced Amount of Feces	5/5 Males, 5/5 Females
	Day 1	Discolored (Blue) Feces	5/5 Males, 5/5 Females
	Day 1	Staining (Blue) of the Skin of the Tail	5/5 Males, 1/5 Females
250	Days 1-2	Staining (Blue) of the Inguinal Hair	1/5 Females
	Day 2	Death	1/5 Males
	Day 2	Slight Weakness	1/5 Females
	Day 2	Reduced Amount of Feces	1/4 Males, 1/5 Females
	Day 2	Staining (Blue) of the Skin of the Tail	4/4 Males
	Day 2	Discolored (Blue) Feces	5/5 Females
	Day 2	Dehydration	1/5 Females
Days 2-3	Staining (Blue) of the Inguinal Hair	4/4 Males	
Days 2-3	Discolored (Blue) Feces	4/4 Males	
250	Day 3	Death	1/5 Females
	Days 3-4	Reduced Amount of Feces	4/4 Males
	Days 3-4	Staining (Blue) of the Skin of the Tail	3/4 Males
	Days 3-6	Discolored (Blue) Feces	4/4 Females
250	Day 4	Discolored (Blue) Feces	3/4 Males
	Day 4	Reduced Amount of Feces	1/4 Females
	Days 4-8	Staining (Blue) of the Inguinal Hair	2/4 Males

Table continued on next page

**RESULTS, Cont.****TABLE OF CLINICAL OBSERVATIONS**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
250	Day 5 Day 5 Day 5 Day 5 Days 5-6	Blue Diarrhea Discolored (Blue) Feces Reduced Amount of Feces Porphyrin Staining of the Facial Hair Dehydration	3/4 Females 4/4 Males 2/4 Males, 3/4 Females 1/4 Males 1/4 Males, 1/4 Females
250	Day 6 Day 6 Day 6 Day 6 Day 6 Day 6	Normal Reduced Amount of Feces Discolored (Blue) Feces Severe Weakness Blue Diarrhea Staining (Blue) of the Skin of the Tail	1/4 Males 3/4 Males, 3/4 Females 2/4 Males 1/4 Females 1/4 Females 2/4 Males
250	Day 7 Day 7 Day 7 Days 7-8 Days 7-8 Days 7-14	Death Normal Reduced Amount of Feces Reduced Amount of Feces Discolored (Blue) Feces Staining (Blue) of the Skin of the Tail	1/4 Females 1/4 Males, 2/3 Females 1/3 Females 1/4 Males 3/4 Males, 1/3 Females 1/4 Males
250	Day 8	Normal	1/4 Males, 2/3 Females
250	Days 9-14 Days 9-14	Normal Staining (Blue) of the Inguinal Hair	3/4 Males, 3/3 Females 1/4 Males

Table continued on next page

**RESULTS. Cont.****TABLE OF CLINICAL OBSERVATIONS. Cont.**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
500	Day 0 Day 0	Blue Diarrhea Staining (Blue) of the Inguinal Hair	2/5 Males, 5/5 Females 2/5 Males, 4/5 Females
500	Day 1 Day 1 Day 1 Day 1 Day 1 Day 1 Day 1	Death Slight Weakness Moderate Weakness Staining (Blue) of the Inguinal Hair Reduced Amount of Feces Discolored (Blue) Feces Staining (Blue) of the Skin of the Tail	1/5 Females 2/5 Males, 2/4 Females 1/4 Females 5/5 Males, 4/4 Females 5/5 Males, 4/4 Females 5/5 Males, 4/4 Females 5/5 Males, 1/4 Females
500	Day 2 Day 2 Day 2 Day 2 Day 2 Day 2 Day 2 Day 2 Days 2-3 Day 2 Days 2-3 Day 2 Days 2-3	Absence of Feces Reduced Amount of Feces Staining (Blue) of the Skin of the Tail Staining (Blue) of the Inguinal Hair Staining (Yellow) of the Inguinal Hair Porphyrin Staining of the Facial Hair Moderate Weakness Dehydration Dehydration Discolored (Blue) Feces Discolored (Blue) Feces Slight Weakness Slight Weakness	2/4 Females 1/5 Males, 1/4 Females 4/5 Males, 2/4 Females 5/5 Males, 3/4 Females 1/4 Females 1/4 Females 1/4 Females 2/4 Females 1/5 Males 2/4 Females 5/5 Males 1/4 Females 1/5 Males
500	Day 3 Day 3 Day 3 Day 3 Day 3 Days 3-6 Days 3-6 Days 3-9 Days 3-4 Days 3-9	Death Slight Weakness Reduced Amount of Feces Dehydration Staining (Blue) of the Skin of the Tail Staining (Blue) of the Skin of the Tail Discolored (Blue) Feces Discolored (Blue) Feces Staining (Blue) of the Inguinal Hair Staining (Blue) of the Inguinal Hair	1/4 Females 1/5 Males, 1/3 Females 5/5 Males, 1/3 Females 1/3 Females 2/3 Females 3/5 Males 3/3 Females 5/5 Males 3/3 Females 3/5 Males

Table continued on next page

**RESULTS. Cont.****TABLE OF CLINICAL OBSERVATIONS. Cont.**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
500	Day 4 Day 4 Day 4 Days 4-5 Days 4-5 Days 4-11	Reduced Amount of Feces Staining (Blue) of the Inguinal Hair Staining (Blue) of the Skin of the Tail Blue Diarrhea Dehydration Staining (Blue) of the Abdominal Hair	4/5 Males, 1/3 Females 3/3 Females 1/3 Females 1/5 Males 2/5 Males 1/3 Females
500	Day 5 Day 5 Days 5-6 Days 5-6 Days 5-6	Reduced Amount of Feces Porphyrin Staining of the Facial Hair Dehydration Staining (Blue) of the Inguinal Hair Staining (Blue) of the Skin of the Tail	3/5 Males, 3/3 Females 2/5 Males 2/3 Females 2/3 Females 2/3 Females
500	Day 6 Day 6 Day 6 Day 6 Days 6-8	Slight Weakness Porphyrin Staining of the Facial Hair Dehydration Staining (Blue) of the Skin of the Tail Reduced Amount of Feces	1/5 Males 1/5 Males 1/5 Males 2/5 Males 5/5 Males, 3/3 Females
500	Day 7 Day 7 Day 7 Day 7 Days 7-10 Days 7-8 Days 7-9 Days 7-8 Day 7 Day 7	Slight Weakness Moderate Weakness Discolored (Blue) Feces Dehydration Dehydration Discolored (Brown) Urine Staining (Blue) of the Skin of the Tail Staining (Blue) of the Inguinal Hair Lack of Feces Reduced Amount of Feces	3/5 Males, 3/3 Females 2/5 Males 2/3 Females 3/5 Males 3/3 Females 3/5 Males 1/5 Males, 1/3 Females 1/3 Females 1/3 Females 2/3 Females

Table continued on next page

**RESULTS. Cont.****TABLE OF CLINICAL OBSERVATIONS. Cont.**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
500	Days 8-9 Day 8 Days 8-10 Days 8-9 Days 8-11 Days 8-11	Slight Weakness Moderate Weakness Moderate Weakness Dehydration Discolored (Blue) Feces Reduced Amount of Feces	1/3 Females 5/5 Males 2/3 Females 5/5 Males 3/3 Females 3/3 Females
500	Day 9 Day 9 Day 9 Days 9-11	Slight Weakness Moderate Weakness Reduced Amount of Feces Staining (Blue) of the Inguinal Hair	4/5 Males 1/5 Males 4/5 Males 3/3 Females
500	Day 10 Days 10-11 Day 10 Day 10 Days 10-11	Severe Weakness Severe Weakness Slight Weakness Blue Diarrhea Staining (Blue) of the Facial Hair Reduced Amount of Feces Discolored (Blue) Feces Dehydration Staining (Blue) of the Inguinal Hair Staining (Blue) of the Skin of the Tail Death Alopecia of the Skin of the Abdomen	1/5 Males 1/3 Females 3/5 Males 1/5 Males 1/5 Males 3/5 Males 3/5 Males 4/5 Males 4/5 Males 2/3 Females 1/5 Males 1/3 Females
500	Day 11 Day 11 Day 11 Day 11 Days 11-12 Day 11 Day 11 Days 11-13 Days 11-13 Days 11-14	Discolored (Brown) Urine Staining (Blue) of the Inguinal Hair Staining (Blue) of the Skin of the Tail Slight Weakness Slight Weakness Moderate Weakness Dehydration Dehydration Reduced Amount of Feces Discolored (Blue) Feces	1/4 Males 3/4 Males 1/3 Females 1/3 Females 3/4 Males 1/3 Females 2/3 Females 2/4 Males 2/4 Males 2/4 Males

Table continued on next page

**RESULTS. Cont.****Clinical Observations. Cont.****TABLE OF CLINICAL OBSERVATIONS. Cont.**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
500	Day 12	Death	2/3 Females
	Day 12	Staining (Blue) of the Inguinal Hair	2/4 Males, 1/1 Females
	Day 12	Reduced Amount of Feces	1/1 Females
	Day 12	Dehydration	1/1 Females
	Days 12-13	Discolored (Blue) Feces	1/1 Females
	Days 12-14	Slight Weakness	1/1 Females
500	Days 12-14	Alopecia of the Skin of the Abdomen	1/1 Females
	Day 13	Slight Weakness	1/4 Males
	Day 13	Moderate Weakness	1/4 Males
500	Days 13-14	Staining (Blue) of the Inguinal Hair	1/4 Males
	Day 14	Slight Weakness	2/4 Males
	Day 14	Reduced Amount of Feces	1/4 Males
500	Day 14	Dehydration	1/4 Males

**RESULTS, Cont.****Clinical Observations, Cont.****TABLE OF CLINICAL OBSERVATIONS, Cont.**

<b>DOSE (mg/kg)</b>	<b>TIME</b>	<b>CLINICAL SIGNS</b>	<b>NUMBER OF ANIMALS AFFECTED</b>
2000	Day 0	Blue Diarrhea Staining (Blue) of the Inguinal Hair Staining (Blue) of the Skin of the Tail	3/5 Males, 5/5 Females 3/5 Males, 5/5 Females 3/5 Males, 5/5 Females
2000	Day 1 Day 1 Days 1-2 Days 1-2 Days 1-2 Days 1-2 Days 1-5	Death Blue Diarrhea Moderate Weakness Severe Weakness Staining (Blue) of the Inguinal Hair Staining (Blue) of the Skin of the Tail Staining (Blue) of the Inguinal Hair	4/5 Males, 4/5 Females 1/1 Males, 1/1 Females 1/1 Males 1/1 Females 1/1 Females 1/1 Females 1/1 Males
2000	Day 2	Reduced Amount of Feces Dehydration	1/1 Females 1/1 Females
2000	Days 2-5	Reduced Amount of Feces Staining (Blue) of the Skin of the Tail	1/1 Males 1/1 Males
2000	Day 3	Slight Weakness Death	1/1 Males 1/1 Females
2000	Days 3-4	Discolored (Blue) Feces	1/1 Males
2000	Days 4-5	Severe Weakness Dehydration Porphyrin Staining of the Facial Hair Rough Haircoat	1/1 Males 1/1 Males 1/1 Males 1/1 Males
2000	Day 6	Death	1/1 Males

**RESULTS. Cont.****Body Weights**

At the 2000 mg/kg dose level, all animals died between Day 1 and Day 6 of the study. Therefore, only initial body weights were recorded for this dose group. A body weight loss was recorded for all eight surviving animals at the 500 mg/kg dose level and for three of seven surviving animals at the 250 mg/kg dose level during the first week of the study. During the second week of the study, a body weight loss was also recorded for a single animal of each sex at the 500 mg/kg dose level. All animals in the 125 mg/kg dose group which survived gained weight during both weeks of the study.

**Individual Body Weights****TABLE OF INDIVIDUAL BODY WEIGHTS - MALES AND FEMALES**

<b>DOSE (mg/kg)</b>	<b>ANIMAL NUMBERS and SEX</b>	<b>START BODY WEIGHT (GRAMS)</b>	<b>TERMINAL WEIGHT (GRAMS)- DAY OF DEATH</b>
2000	911 Male	177	167 - Day 1
2000	912 Male	204	134 - Day 6
2000	913 Male	207	203 - Day 1
2000	914 Male	192	181 - Day 1
2000	915 Male	184	169 - Day 1
2000	916 Female	181	172 - Day 1
2000	917 Female	170	156 - Day 1
2000	918 Female	155	144 - Day 1
2000	919 Female	175	153 - Day 3
2000	920 Female	172	157 - Day 1

**RESULTS, Cont.****Individual Body Weights, Cont.****TABLE OF INDIVIDUAL BODY WEIGHTS - MALES**

DOSE (mg/kg)	ANIMAL NUMBER	INDIVIDUAL BODY WEIGHTS (grams)		
		DAY 0	DAY 7	DAY 14 (Terminal)
125	241	223	302	364
125	242	229	293	348
125	243	231	320	387
125	244	227	292	344
125	245	236	281	354
250	246	203	209	305
250	247	227	Died Day 2	(190)
250	248	224	210	321
250	249	221	270	337
250	250	228	203	300
500	251	220	169	260
500	252	220	174	Died Day 10 (141)
500	253	218	202	272
500	254	230	210	177
500	255	217	213	222

**RESULTS. Cont.****Individual Body Weights. Cont.****TABLE OF INDIVIDUAL BODY WEIGHTS - FEMALES**

DOSE (mg/kg)	ANIMAL NUMBER	INDIVIDUAL BODY WEIGHTS (grams)		
		DAY 0	DAY 7	DAY 14 (Terminal)
125	256	188	224	257
125	257	202	243	271
125	258	185	232	259
125	259	182	Died Day 3	(148)
125	260	173	205	218
250	261	185	220	255
250	262	182	Died Day 3	(151)
250	263	191	218	264
250	264	183	168	253
250	265	182	Died Day 7	(133)
500	266	185	153	Died Day 12 (123)
500	267	171	149	Died Day 12 (120)
500	268	185	148	165
500	269	188	Died Day 1	(179)
500	270	203	Died Day 3	(172)

**RESULTS. Cont.**

**Necropsy and Histopathology Findings**

Treatment-related changes observed at necropsy included the test material in the trachea, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum; moderate blue discoloration by the test material in the mucosa of the trachea, glandular and non-glandular stomach, duodenum, jejunum, ileum, cecum, colon, and rectum; blue discoloration by the test material of the skin of the feet and tail, and the inguinal and facial hair; the hair of the face discolored red; abdominal adipose tissue and duodenal mucosa were discolored green; a small thymus, spleen, and accessory sex glands; enlarged adrenal glands; a decrease or absence of abdominal adipose tissue; hemorrhage in the lungs; nasal porphyrin discharge; alopecia of the abdominal skin; inguinal hair wet by urine; and a dry urine stain present on the inguinal and abdominal hair. Small (1-2 mm in diameter) raised, round, white structures with indented centers were observed in the non-glandular gastric mucosa of a single rat.

Enlarged adrenal glands from two rats at the 500 mg/kg dose level and the stomach from a single rat at the 500 mg/kg dose level were processed for microscopic examination. The adrenal glands of both rats were histologically normal. The stomach showed moderate hyperkeratosis and minor acanthosis of the non-glandular gastric mucosa. Lesions in the non-glandular gastric mucosa were characterized by a thickened keratin layer, which was otherwise normal, and acanthosis, or increased thickness of stratum spinosum (prickle cell layer). Above the thickened keratin layer there were two basket-like structures composed of tightly woven keratin strands. These structures were initially diagnosed as ulcers on gross examination. Hyperkeratosis and acanthosis of the non-glandular gastric mucosa are usually provoked by irritation. The glandular gastric mucosa was normal. A detailed record of the incidence and severity of all gross abnormalities and histological findings are presented in computer-generated tables which are included in the appended pathology report.

## DATA ANALYSIS

The LD<sub>50</sub> was obtained using the method of Weil (1952). The results were as follows:

LD <sub>50</sub> for male rats:	794 mg/kg (95% C.I. = 471 - 1339 mg/kg)
LD <sub>50</sub> for female rats:	315 mg/kg (95% C.I. = 133 - 748 mg/kg)
LD <sub>50</sub> for both sexes combined:	583 mg/kg (95% C.I. = 365 - 933 mg/kg)

No dose/mortality curve was constructed since graphs become statistically useful only with the use of large numbers of animals and dose groups.

## DISCUSSION AND INTERPRETATION

The acute oral LD<sub>50</sub> for this test material was calculated to be 794 mg/kg for male rats and 315 mg/kg for female rats. The acute oral LD<sub>50</sub> calculated by combining the male and female mortality data was 583 mg/kg.

Mortality was first observed on the day following dosing and continued to Day 12 of the 14-day observation period. The majority of abnormal clinical signs were due to staining or discoloration by the test material. These included blue diarrhea and blue staining or discoloration of the feces; skin of the tail; and hair of the abdomen, face and inguinal area. Other abnormal clinical signs included slight to severe weakness, a reduced amount or lack of feces, discolored (brown) urine, staining (yellow) of the inguinal hair, dehydration, porphyrin staining of the facial hair, and rough haircoat. Alopecia of the abdomen was observed for a single female at the 500 mg/kg dose level. A body weight loss was recorded for all eight surviving animals at the 500 mg/kg dose level and for three of seven surviving animals at the 250 mg/kg dose level during the first week of the study. During the second week of the study, a body weight loss was also recorded for a single animal of each sex at the 500 mg/kg dose level. All animals in the 125 mg/kg dose group which survived gained weight during both weeks of the study.

The cause of death for rats which died after exposure to the test material was not determined. Lesions observed at necropsy in rats on this study could be classified into four categories: lesions observed in the majority of rats, lesions observed in the high-dose group rats, lesions that are considered to be secondary to loss of body weight and/or stress, and sporadic lesions which are observed in few rats. In many rats, the test material was present in the gastrointestinal tract; and blue discoloration was observed on the mucosa of the gastrointestinal tract, skin of the feet, and facial or inguinal hair. In the high-dose group rats, test material

### DISCUSSION AND INTERPRETATION. Cont.

was present in the trachea of one of five male and four of five female rats, and lung hemorrhage was observed in all five male rats and four of five female rats. Lesions which could be ascribed to loss of body weight and/or stress included small thymuses, small spleens, small male accessory sex glands, and reduced abdominal adipose tissue. Other lesions were determined to be incidental changes.

### CONCLUSION

Based on the oral LD<sub>50</sub> calculated from the combined mortality data, the test material was classified as slightly toxic in rats according to the criteria set forth by Hodge and Sterner (1949) and classified as harmful if swallowed as defined in the 18th Adaptation on the EC Classification, Packaging, and Labelling of Dangerous Substances.

### DATA STORAGE

All test results presented in this report are supported by raw data which are maintained in the archives of the Health and Environment Laboratories, Eastman Kodak Company.

### REFERENCES

- Hodge, H.C. and Sterner, J.H. (1949). Tabulation of toxicity classes. *Am. Indust. Hyg. Quart.*, 10:93-96.
- Weil, C.S. (1952). Tables of convenient calculations of medium-effective dose (LD<sub>50</sub> or ED<sub>50</sub>) and instructions in their use. *Biometrics*, 8:249-263.

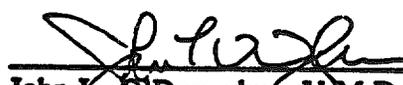
**SIGNATURE PAGE**

  
\_\_\_\_\_  
Kenneth P. Shepard, B.S.  
Study Director

4 / 12 / 95  
\_\_\_\_\_  
Month/Day/Year

  
\_\_\_\_\_  
Douglas C. Topping, Ph.D.  
Unit Director, Mammalian Toxicology Section

April 11, 1995  
\_\_\_\_\_  
Month/Day/Year

  
\_\_\_\_\_  
John L. O'Donoghue, V.M.D., Ph.D.  
Director, Health and Environment Laboratories

4 / 12 / 95  
\_\_\_\_\_  
Month/Day/Year

**Best Available Copy**