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Submitting Organization			
EASTMAN KODAK CO			
Contractor			
Document Title			
INITIAL SUBMISSION: LETTER FR EASTMAN KODAK CO TO USEPA RE ACUTE/SUBACUTE ORAL & DERM TOX TESTS W/DISUBSTITUTED MORPHOLINYL-2-CYCLOPEN-1-ONE, W/ATTCHMTS & DATED 111699 (SANITIZED)			
Chemical Category			
DISUBSTITUTED MORPHOLINYL-2-CYCLOPEN-1-ONE (CONFIDENTIAL)			

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INITIAL SUB- MISSION

B 02

A 03

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November 16, 1999

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Document Control Office (7407)
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street
Washington, DC 20460-001
ATTN: TSCA Section 8(e) Coordinator

Company Sanitized

Dear Sir or Madam:

Subject: Report submitted in accordance with U. S. Environmental Protection Agency
Statement of Interpretation and Enforcement policy; Notification of Substantial
Risk-Section 8(e) TSCA.

The following information is submitted in accordance with the above statement. The
submission pertains to two structurally similar compounds, both identified by the generic name
disubstituted morpholinyl-2-cyclopenten-1-one. Compound 1 is

[CAS # NA] and Compound 2 is

[CAS # NA]. These compounds are being submitted

because of neurological effects observed in acute oral toxicity screening studies in rats.

In each study, single oral doses of 2000, 1000, 600, 300, or 100 mg/kg of test substance were
administered by gavage to fasted rats (2 females/ dose level). Subsequently, each surviving
female at the 1000, 600, 300, and 100 mg/kg dose levels received a daily dose identical to the
initial dose for three consecutive days to provide a preliminary assessment of subacute
toxicity.

For Compound 1, mortality was noted for all animals which received a single oral dose of 1000
or 2000 mg/kg of the test substance. Both animals in the 600 mg/kg/day dose group and one
animal in the 300 mg/kg/day dose group died after receiving two oral doses. The remaining
animal in the 300 mg/kg/day dose group died after receiving three oral doses. Flattened posture
and retropulsion were observed for one rat in the 1000 mg/kg dose group. No other abnormal
clinical signs were observed during the study with this compound.

For Compound 2, mortality was noted for all animals which received a single oral dose of 600,
1000, or 2000 mg/kg of the test substance. One animal at the 600 mg/kg dose level survived to
the morning following dosing at which time it had a swollen face and loss of righting reflex.
One animal assigned to the 300 mg/kg/day dose level displayed signs of circling, head search,
and retropulsion on the day following the fourth dose.

R. Hays Bell, Ph D., Director, Health, Safety, and Environment
Vice President, Eastman Kodak Company
Rochester, NY 14652-6256 • 716-722-5036 • FAX 716-722-0239

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The NOEL (No Observable Effect Level) for animals receiving repeated doses of either test compound under the conditions of the study was 100 mg/kg/day.

These chemicals are research and development chemicals.

We have claimed parts of this letter and these enclosures as confidential (denoted by encircling). A public file version of this submission is also enclosed. Information to support our claim of confidentiality can be found in the attachment to this letter.

Sincerely,



R. Hays Bell
(716) 722-5036

RHB:JAF

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SUPPORT INFORMATION FOR CONFIDENTIALITY CLAIMS

Specific information is provided below:

- 1. **Is your company asserting this confidential business information (CBI) claim on its own behalf? If the answer is no, please provide company name, address and telephone number of entity asserting claim.**

We are asserting the chemical identity confidentiality claim on our own behalf.

- 2. **For what period do you assert your claim(s) of confidentiality? If the claim is to extend until a certain event or point in time, please indicate that event or time period. Explain why such information should remain confidential until such point?**

These chemicals are currently being investigated for use in a photographic article. Since these chemicals are not currently in commercial products, competitors are not aware of their identity, and therefore, confidentiality should be granted indefinitely.

- 3. **Has the information that you are claiming as confidential been disclosed to any other governmental agency, or to this Agency at any other time? Identify the Agency to which the information was disclosed and provide the date and circumstances of the same. Was the disclosure accompanied by a claim of confidentiality? If yes, attach a copy of said document reflecting the confidentiality agreement.**

To the best of our knowledge, the information being claimed as confidential has not been disclosed to any other governmental agency or to the U. S. Environmental Protection Agency prior to this submission.

- 4. **Briefly describe any physical or procedural restrictions within your company relating to the use and storage of the information you are claiming CBI.**

Standard methods of protecting confidential business information have been used including classification of documents, limited and controlled distribution of documents, secure storage areas for documents, transmission of documents by certified mail or courier service, employee education programs, and special security procedures for computer files. In addition, employees upon joining the company sign an "Employee's Agreement", part of which states that they will not, either during their employment or thereafter, disclose to anyone outside the company or make use of confidential business information that they may acquire during their employment with the company. Confidential information that is distributed outside the company is only done so under the terms of a confidentiality agreement.

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5. **If anyone outside your company has access to any of the information claimed CBI, are they restricted by confidentiality agreement(s)? If so, explain the content of the agreement(s).**

These chemicals are research and development chemicals manufactured in Eastman Kodak Company. No one outside of Eastman Kodak Company has access to this information or is aware of these chemicals.

6. **Does the information claimed as confidential appear or is referred to in any of the following:**

- a. **Advertising or promotional material for the chemical substance or the resulting end product;**
- b. **Material safety data sheets or other similar materials (such as technical data sheets) for the substance or resulting end product (include copies of this information as it appears when accompanying the substance and/or product at the time of transfer or sale);**
- c. **Professional or trade publication; or**
- d. **Any other media or publications available to the public or to your competitors.**

If you answered yes to any of the above, indicate where the information appears, include copies, and explain why it should nonetheless be treated as confidential.

The information claimed as confidential does not appear in advertising or promotional materials, professional or trade publications, or any other media available to the public or to our competitors. Although this information is available to employees through the use of material safety data sheets, it is protected through our standard methods of protecting confidential business information described in item 4 above.

7. **Has EPA, another federal agency, or court made any confidentiality determination regarding information associated with this substance? If so, provide copies of such determinations.**

To the best of our knowledge, there have been no confidentiality determinations by EPA, any federal agency, or any court in connection with this information.

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8. Describe the substantial harmful effects that would result to your competitive position if the CBI information is made available to the public? In your answer, explain the causal relationship between disclosure and any resulting substantial harmful effects. Consider in your answer such constraints as capital and marketing cost, specialized technical expertise, or unusual processes and your competitors access to your customers. Address each piece of information claimed CBI separately.

Disclosure of the specific identities of these chemicals would cause substantial harm by allowing competitors to deduce at an earlier time and without incurring their own research and development costs that these chemicals have advantages over other chemicals now in use. This would place them in the favorable position of being able to use this innovation, which is the result of our research and development, in their products at an earlier time while incurring little or no research or development costs of their own.

Disclosure of the identities of these chemicals would cause substantial harm by allowing a competitor, in conjunction with other information, to more easily analyze for either chemical and then determine the preferred level of it in the article, to deduce how it is involved in the process, and the performance advantage that is achieved by its use.

Disclosure of the specific chemical identities could also cause harm by providing insight to competitors about the type of new and improved photographic products we will be introducing domestically and internationally.

This harm would be expected to occur even if the manufacturer's identity were held confidential because competitors would associate either of these chemicals with similar compounds already used in the manufacture of photographic products.

9. Has the substance been patented in the U.S. or elsewhere? Is a patent for the substance currently pending?

To the best of our knowledge, there is no issued or pending patent for these substances in the U.S. or elsewhere.

10. Is this substance/product commercially available and if so, for how long has it been available on the commercial market?

a. If on the commercial market, are your competitors aware that the substance is commercially available in the U.S.?

b. If not already commercially available, describe what stage of research and development (R&D) the substance is in, and estimate how soon a market will be established.

Document Control Office --6

- c. **What is the substance used for and what type of product(s) does it appear in?**

These substances are being investigated under research and development as possible photographic doctors to improve photographic performance of film and paper. They are being manufactured by Eastman Kodak Company and are not commercially available. Research and development for these chemicals is in the early stages. If chosen for use in commercial products, commercial availability could be in mid 2000.

11. **Describe whether a competitor could employ reverse engineering to identically recreate the substance.**

It is possible that a competitor, knowledgeable in this type of chemistry, could analyze our products and employ reverse engineering to identically recreate either of these substances. Parts of either substance are well known photographic building blocks and, with the identity of the substance, a competitor could deduce the preferred manufacturing process.

12. **Do you assert that disclosure of this information you are claiming CBI would reveal:**

- a. **confidential processes used in manufacturing the substance;**
- b. **if in a mixture, the actual portions of the substance in the mixture; or**
- c. **information unrelated to the effects of the substance on human health or the environment?**

If your answer to any of the above questions is yes, explain how such information would be revealed.

Disclosure of either chemical could disclose part of the process (reaction sequence) used to manufacture either chemical. Competitors, in conjunction with other information, could deduce the function of either chemical, the type of article involved and part of the process used to manufacture the article. This would place them in the favorable position of being able to incorporate this innovation, which is the result of our research and development effort, in their products at an earlier time while incurring little or no research or development costs of their own.

Disclosure of either chemical identity could disclose the portion of the coated article comprised of the final chemical. This would allow competitors to more easily analyze for either chemical and then determine the preferred level of it in the article.

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Disclosure of the identity of either chemical is not necessary to interpret any of the health and safety studies because the generic name reveals the toxicologically significant portions of the chemical.

13. **Provide the Chemical Abstract Service Registry Number for the product, if known. Is your company applying for a CAS number now or in the near future? If you have applied for a CAS number, include a copy of the contract with CAS.**

The CAS Registry Numbers assigned to either substance are: NA.

14. **Is the substance or any information claimed CBI the subject of FIFRA regulation or reporting? If so, explain.**

To the best of our knowledge, the substance and other information claimed CBI are not the subject of FIFRA regulation or reporting.

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FINAL REPORT

COMPOUND 1 Disubstituted morpholinyl-2-cyclopenten-1-one

HAEL No.:

RAN:

KAN:

**ACUTE/SUBACUTE ORAL TOXICITY AND DERMAL IRRITATION
SCREENING TEST IN THE RAT**

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 Kodak Company

STUDY COMPLETION DATE

November 1, 1999

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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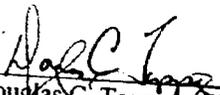
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Kenneth P. Shepard, B.S.
Study Director

11/1/99
Month/Day/Year



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

Oct 29, 1999
Month/Day/Year

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ABSTRACT

HAEL No.:

RAN:

KAN:

**ACUTE/SUBACUTE ORAL TOXICITY AND DERMAL IRRITATION
SCREENING TEST IN THE RAT**

An acute toxicity screening study was conducted by administering single oral doses of 2000, 1000, 600, 300, or 100 mg of test substance per kg of body weight by gavage to fasted rats (2 females/dose level). Approximately four hours after administration of these doses to the fasted animals, the rats were fed and allowed *ad libitum* access to feed for the remainder of the study. Subsequently, each female at the 1000, 600, 300, and 100 mg/kg dose levels received a daily dose identical to the initial dose for three consecutive days to provide a preliminary assessment of subacute toxicity. To assess the potential of the test substance to cause dermal irritation, one of the two rats assigned to the 100 mg/kg dose group was additionally exposed to 0.5 gram of the test substance topically for a period of four hours.

Mortality was noted for all animals which received a single oral dose of 1000 or 2000 mg/kg of the test substance. One animal at the 1000 mg/kg dose level had a flattened posture and demonstrated retropulsion on the morning following dosing. In addition, both animals assigned to the 600 mg/kg/day dose group and one of two animals at the 300 mg/kg/day dose level died after receiving two oral doses. The remaining animal in the 300 mg/kg/day dose group died after receiving three oral doses. The single-dose oral LD₅₀ was less than 1000 mg/kg. Both animals in the 100 mg/kg/day dose group which survived to termination of the screen gained weight during the observation period. The NOEL (No Observable Effect Level) for animals receiving repeated doses (four daily) under the conditions of this study was 100 mg/kg/day.

In assessing dermal irritation, no signs of erythema or edema were evident at the application site of the single female which received topical exposure to the test substance.

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.
Report Author:	Kenneth P. Shepard, B.S.

Sponsor

Eastman Kodak Company

Test Substance Characterization

Test Substance Name:	
CAS Reg. No.:	Not Available
HAEL No.:	
KAN:	
SRID No.:	
Physical State and Appearance:	Solid, Rust-colored crystal
Source of Test Substance:	Eastman Kodak Company

Study Dates

Study Initiation Date:	October 18, 1999
Experimental Start Date:	October 18, 1999
Experimental Completion Date:	October 22, 1999

PURPOSE

The purpose of this series of exposures was to estimate the acute and repeated-dose oral toxicity, and the dermal irritation potential of the test substance in the rat.

MATERIALS AND METHODS

Test system

Ten female Sprague-Dawley rats (CrI:CD(SD)BR) obtained from Charles River Laboratories, Stone Ridge (Kingston), NY were randomly assigned so that each dose group included two animals. Rats were chosen for this study because they are a common representative species for toxicity studies.

Husbandry

Housing

Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-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 18.5 to 24.6 °C and 38.7 to 60.4% relative humidity. A photoperiod of 12 hours light from approximately 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.

E

Husbandry, continued

Feed

Certified Rodent Diet (PMI #5002, pelleted) was available *ad libitum*. Feed containers were cleaned prior to use and at termination of the study. 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 series of exposures was initiated as a screening study for which there are no published guidelines.

Test Standards

While this screening study was performed using methods consistent with domestic and international good laboratory practice (GLP) standards, it was not audited for compliance with these standards.

Randomization

The procedure for including animals in the screening study was to randomly select and assign animals from the same shipment to the different dose levels. Randomization of animals to dose level groups was completed using computer-generated random number lists. After assignment of animals to the dose levels, body weights were determined to ensure that variation in individual body weights did not exceed 20% of the mean weight.

Experimental Design, continued

Dose Levels and Test Substance Exposure

Groups of two fasted female rats were administered an initial oral dose of 2000, 1000, 600, 300, or 100 mg of the test substance/kg of body weight on Day 0. Approximately four hours after dosing, the animals were offered *ad libitum* access to feed for the remainder of the study. All surviving rats assigned to the 1000, 600, 300, and 100 mg/kg dose groups subsequently received three additional daily doses of the test substance on Days 1, 2, and 3 of the study.

Approximately one hour after the second oral dose of 100 mg/kg, one of the two females assigned to this dose level received an additional exposure of 0.5 gram of the test substance topically. For the topical exposure, a single dose of the test substance was placed against the clipped skin of the back using a semi-occlusive wrap for a period of four hours.

There was no control group assigned to this study.

Preparation of Test Substance in the Vehicle

For oral exposure to the test substance, a single concentration of the test substance was prepared. The test substance was administered at a concentration of 200 mg/mL using corn oil as the vehicle. The preparation was refrigerated between dosing periods. For the topical exposure, the test substance was administered as a solid thoroughly moistened with water. The test substance purity was not determined, and the dose preparation was not analyzed for concentration, homogeneity, or stability.

Body Weights

Body weights were collected on Days 0 (prior to treatment), 2, and 4.

Observations

The observation period was five days and extended from the time of the first treatment on Day 0 until the morning of Day 4. Animals were observed twice a day. On Day 0, observations were made during the first hour after treatment and again in the afternoon. On subsequent days, the animals were observed once in the morning (prior to treatment) and approximately six hours later in the afternoon, except on Day 4 when the screening study was terminated after the morning observations. All animals were observed for mortality, morbidity, and general activity levels. Observations were recorded as either

Experimental Design, continued

"A" for animals alive and active, "M" for animals in a moribund condition, or "D" for animals found dead. Morning observations also included any unusual response with respect to body position, activity level, and coordination of movement.

In addition, the skin application site for the rat that received the topical exposure was observed daily for signs of irritation. The most severely affected area within the site of application of the test substance was scored for erythema and edema. The dermal irritation scale, published by Draize, et al. (1944) was used for scoring reactions.

Necropsy

Animals were euthanatized by inhalation of carbon dioxide on Day 4 after the morning observations. Necropsies were not performed at the completion 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.

Statistical Procedures

No statistical procedures were conducted on the data collected during this study.

Standard Operating Procedure and Protocol Deviations

There were no standard operating procedure or protocol deviations during this study.

Test Substance Exposure**RESULTS**

The dose level, dose volume in mL/kg, number of animals dosed, animal numbers, and actual volumes of the test substance administered to each animal are listed in Table 1.

TABLE 1 (Exposure Table)

Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Animals	Animal Numbers	Volume (mL) Administered
2000	10.0	2 Females	521	1.54
			522	1.56
1000	5.0	2 Females	523	0.76
			524	0.75
600	3.0	2 Females	525	0.45
			526	0.47
300	1.5	2 Females	527	0.21
			528	0.21
100	0.5	2 Females	529	0.07
			530*	0.07

* Animal received an additional topical exposure of 0.5 gram for a period of 4 hours.

Mortality

The number of animals dosed, the number of deaths, and the day of death are listed in Table 2.

TABLE 2 (Mortality Table)

Dose (mg/kg)	Number of Rats	Number of Deaths	Time of Death
2000	2	2	Day 0 & Day 1
1000	2	2	Day 0 & Day 1
600	2	2	Day 1 & Day 2
300	2	2	Day 2 & Day 3
100	2	0	Not Applicable

General Observations

General observations were recorded either "A" for animals alive and active, "M" for animals in a moribund condition, or "D" for animals found dead. Any unusual response was also noted during morning observations. The time of each observation and the number of animals involved at each dose level are listed in Table 3.

TABLE 3 (Table of Observations)

Dose Level (mg/kg)	Animal Number	Observations (A=Alive, M=Moribund, and D=Dead)								
		Day 0		Day 1		Day 2		Day 3		Day 4
		AM	PM	AM	PM	AM	AM	AM	PM	AM
2000	521	D	---	---	---	---	---	---	---	---
	522	A	A	D	---	---	---	---	---	---
1000	523	A	A	α	D	---	---	---	---	---
	524	A	D	---	---	---	---	---	---	---
600	525	A	A	A	D	---	---	---	---	---
	526	A	A	A	A	D	---	---	---	---
300	527	A	A	A	A	D	---	---	---	---
	528	A	A	A	A	A	A	---	---	---
100	529	A	A	A	A	A	A	D	---	---
	530	A	A	A	A	A	A	A	A	A

α Flattened position and retropulsion were noted for this animal.

Adverse Clinical Signs

Flattened posture and retropulsion were noted for Rat 523 (1000 mg/kg/day) at examinations on the day following the initial dose. No other abnormal clinical signs were noted during this screen.

Observations for Irritation

The application site of the animal exposed to the test substance topically was examined for signs of erythema and edema and the responses scored at 1, 24, 48, and 72 hours after termination of exposure to the test substance. The scores for irritation recorded are presented in Table 4.

TABLE 4 (Table of Irritation Scores)

Animal Number	Response at Application Site (Erythema, Edema) ¹			
	1 Hour	24 Hours	48 Hours	72 Hours
530	0,0	0,0	0,0	0,0

¹ The four-point scale, published by Draize, et al. (1944) was used for scoring dermal irritation.

Body Weights

All surviving animals gained weight from the day of dosing to termination of the screen.

Individual Body Weights

The individual body weights are listed in Table 5.

TABLE 5 (Table of Individual Body Weights (grams))

Dose (mg/kg)	Animal Number	Day 0	Day 2	Day 4
2000	521	154	-----	-----
2000	522	156	-----	-----
1000	523	151	-----	-----
1000	524	149	-----	-----
600	525	149	-----	-----
600	526	155	-----	-----
300	527	143	-----	-----
300	528	146	144	-----
100	529	144	162	173
100	530	137	152	153

DISCUSSION AND CONCLUSION

In this screen, mortality was noted for all animals which received a single oral dose of 1000 or 2000 mg/kg of the test substance. One animal at the 1000 mg/kg dose level had a flattened posture and demonstrated retropulsion on the morning following dosing. Both animals assigned to the 600 mg/kg/day dose group and one of two animals at the 300 mg/kg/day dose level died after receiving two oral doses. The remaining animal in the 300 mg/kg/day dose group died after receiving three oral doses. The single-dose oral LD₅₀ was less than 1000 mg/kg. Both animals in the 100 mg/kg/day dose group which survived to termination of the screen gained weight during the observation period. The NOEL (No Observable Effect Level) for animals receiving repeated doses (four daily) under the conditions of this study was 100 mg/kg/day.

No signs of dermal irritation were evident at the application site of the single female which received a topical exposure to the test substance.

REFERENCES

- Draize, J.H., Woodard, G., and Calvery, H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucus membranes, *J. Pharmacol. Exp. Ther.*, 82:377-390.

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FINAL REPORT

COMPOUND 2 - Disubstituted morpholinyl-2-cyclopenten-1-one

HAEL No.:

RAN:

KAN:

**ACUTE/SUBACUTE ORAL TOXICITY AND DERMAL IRRITATION
SCREENING TEST IN THE RAT**

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 Kodak Company

STUDY COMPLETION DATE

November 1, 1999

B. 11

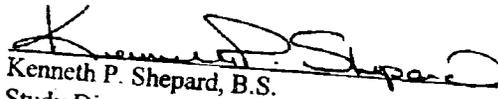
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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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SIGNATURE PAGE


Kenneth P. Shepard, B.S.
Study Director

11/1/99
Month/Day/Year


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

Oct 29, 1999
Month/Day/Year

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ABSTRACT

HAEL No.:

RAN:

KAN:

**ACUTE/SUBACUTE ORAL TOXICITY AND DERMAL IRRITATION
SCREENING TEST IN THE RAT**

An acute toxicity screening study was conducted by administering single oral doses of 2000, 1000, 600, 300, or 100 mg of test substance per kg of body weight by gavage to fasted rats (2 females/dose level). Approximately four hours after administration of these doses to the fasted animals, the rats were fed and allowed *ad libitum* access to feed for the remainder of the study. Subsequently, each female at the 1000, 600, 300, and 100 mg/kg dose levels received a daily dose identical to the initial dose for three consecutive days to provide a preliminary assessment of subacute toxicity. To assess the potential of the test substance to cause dermal irritation, one of the two rats assigned to the 100 mg/kg dose group was additionally exposed to 0.5 gram of the test substance topically for a period of four hours.

Mortality was noted for all animals which received a single oral dose of 600, 1000, or 2000 mg/kg of the test substance. One animal at the 600 mg/kg dose level survived to the morning following dosing at which time it had a swollen face and loss of righting reflex. The single-dose oral LD₅₀ was between 300 and 600 mg/kg. For animals receiving multiple daily doses, the two animals assigned to the 300 mg/kg/day dose level gained weight between Day 0 and Day 2 (18 and 14 grams), but lost weight between Day 2 and Day 4 (7 and 4 grams). One animal assigned to the 300 mg/kg/day dose level displayed signs of circling, head search, and retropulsion on the day following the fourth dose. All animals assigned to the 100 mg/kg/day dose level gained weight from the day of dosing to termination of the screen. The NOEL (No Observable Effect Level) for animals receiving repeated doses (four daily) under the conditions of the study was 100 mg/kg/day.

In assessing dermal irritation, no signs of erythema or edema were evident at the application site of the single female which received topical exposure to the test substance.

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:
Principal Investigator:
Report Author:

Kenneth P. Shepard, B.S.
John W. Mosher, B.S.
Kenneth P. Shepard, B.S.

Sponsor

Eastman Kodak Company

Test Substance Characterization

Test Substance Name:
CAS Reg. No.:
HAEL No.:
KAN:
SRID No.:
Physical State and Appearance:
Source of Test Substance:

Not Available

Solid, Off-white powder
Eastman Kodak Company

Study Dates

Study Initiation Date:
Experimental Start Date:
Experimental Completion Date:

October 18, 1999
October 18, 1999
October 22, 1999

PURPOSE

The purpose of this series of exposures was to estimate the acute and repeated-dose oral toxicity, and the dermal irritation potential of the test substance in the rat.

MATERIALS AND METHODS

Test system

Ten female Sprague-Dawley rats (CrI:CD(SD)BR) obtained from Charles River Laboratories, Stone Ridge (Kingston), NY were randomly assigned so that each dose group included two animals. Rats were chosen for this study because they are a common representative species for toxicity studies.

Husbandry

Housing

Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-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 18.5 to 24.6 °C and 38.7 to 60.4% relative humidity. A photoperiod of 12 hours light from approximately 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, continuedFeed

Certified Rodent Diet (PMI #5002, pelleted) was available *ad libitum*. Feed containers were cleaned prior to use and at termination of the study. 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 DesignTest Procedures

This series of exposures was initiated as a screening study for which there are no published guidelines.

Test Standards

While this screening study was performed using methods consistent with domestic and international good laboratory practice (GLP) standards, it was not audited for compliance with these standards.

Randomization

The procedure for including animals in the screening study was to randomly select and assign animals from the same shipment to the different dose levels. Randomization of animals to dose level groups was completed using computer-generated random number lists. After assignment of animals to the dose levels, body weights were determined to ensure that variation in individual body weights did not exceed 20% of the mean weight.

Experimental Design, continued

Dose Levels and Test Substance Exposure

Groups of two fasted female rats were administered an initial oral dose of 2000, 1000, 600, 300, or 100 mg of the test substance/kg of body weight on Day 0. Approximately four hours after dosing, the animals were offered *ad libitum* access to feed for the remainder of the study. All surviving rats assigned to the 1000, 600, 300, and 100 mg/kg dose groups subsequently received three additional daily doses of the test substance on Days 1, 2, and 3 of the study.

Approximately one hour after the second oral dose of 100 mg/kg, one of the two females assigned to this dose level received an additional exposure of 0.5 gram of the test substance topically. For the topical exposure, a single dose of the test substance was placed against the clipped skin of the back using a semi-occlusive wrap for a period of four hours.

There was no control group assigned to this study.

Preparation of Test Substance in the Vehicle

For oral exposure to the test substance, a single concentration of the test substance was prepared. The test substance was administered at a concentration of 200 mg/mL using corn oil as the vehicle. The preparation was refrigerated between dosing periods. For the topical exposure, the test substance was administered as a solid thoroughly moistened with water. The test substance purity was not determined, and the dose preparation was not analyzed for concentration, homogeneity, or stability.

Body Weights

Body weights were collected on Days 0 (prior to treatment), 2, and 4.

Observations

The observation period was five days and extended from the time of the first treatment on Day 0 until the morning of Day 4. Animals were observed twice a day. On Day 0, observations were made during the first hour after treatment and again in the afternoon. On subsequent days, the animals were observed once in the morning (prior to treatment) and approximately six hours later in the afternoon, except on Day 4 when the screening study was terminated after the morning observations. All animals were observed for mortality, morbidity, and general activity levels. Observations were recorded as either

Experimental Design, continued

"A" for animals alive and active, "M" for animals in a moribund condition, or "D" for animals found dead. Morning observations also included any unusual response with respect to body position, activity level, and coordination of movement.

In addition, the skin application site for the rat that received the topical exposure was observed daily for signs of irritation. The most severely affected area within the site of application of the test substance was scored for erythema and edema. The dermal irritation scale, published by Draize, et al. (1944) was used for scoring reactions.

Necropsy

Animals were euthanatized by inhalation of carbon dioxide on Day 4 after the morning observations. Necropsies were not performed at the completion 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.

Statistical Procedures:

No statistical procedures were conducted on the data collected during this study.

Standard Operating Procedure and Protocol Deviations

There were no standard operating procedure or protocol deviations during this study.

General Observations

General observations were recorded either "A" for animals alive and active, "M" for animals in a moribund condition, or "D" for animals found dead. Any unusual response was also noted during morning observations. The time of each observation and the number of animals involved at each dose level are listed in Table 3.

TABLE 3 (Table of Observations)

Dose Level (mg/kg)	Animal Number	Observations (A=Alive, M=Moribund, and D=Dead)								
		Day 0		Day 1		Day 2		Day 3		Day 4
		AM	PM	AM	PM	AM	AM	AM	PM	AM
2000	511	D	---	---	---	---	---	---	---	---
	512	A	D	---	---	---	---	---	---	---
1000	513	A	D	---	---	---	---	---	---	---
	514	A	D	---	---	---	---	---	---	---
600	515	A	A	D	---	---	---	---	---	---
	516	A	A	α	D	---	---	---	---	---
300	517	A	A	A	A	A	A	A	A	A
	518	A	A	A	A	A	A	A	A	A
100	519	A	A	A	A	A	A	A	A	β
	520	A	A	A	A	A	A	A	A	A

α Loss of righting reflex and swollen face were noted for Rat 516.
 β Circling, head search, and retropulsion were noted for Rat 518.

Adverse Clinical Signs

Loss of righting reflex and swollen face were noted for Rat 516 (600 mg/kg) on the day after the first dose and circling, head search, and retropulsion were noted for Rat 518 (300 mg/kg/day) on the day following the fourth dose. No other abnormal clinical signs were noted during this screen.

Observations for Irritation

The application site of the animal exposed to the test substance topically was examined for signs of erythema and edema and the responses scored at 1, 24, 48, and 72 hours after termination of exposure to the test substance. The scores for irritation recorded are presented in Table 4.

TABLE 4 (Table of Irritation Scores)

Animal Number	Response at Application Site (Erythema, Edema) ¹			
	1 Hour	24 Hours	48 Hours	72 Hours
520	0,0	0,0	0,0	0,0

¹ The four-point scale, published by Draize, et al. (1944) was used for scoring dermal irritation.

Body Weights

For animals assigned to the 300 mg/kg/day dose level, both animals gained weight between Day 0 and Day 2 (18 and 14 grams), but lost weight between Day 2 and Day 4 (7 and 4 grams). All animals assigned to the 100 mg/kg/day dose level gained weight from the day of dosing to termination of the screen.

Individual Body Weights

The individual body weights are listed in Table 5.

TABLE 5 (Table of Individual Body Weights (grams))

Dose (mg/kg)	Animal Number	Day 0	Day 2	Day 4
2000	511	143	-----	-----
2000	512	136	-----	-----
1000	513	142	-----	-----
1000	514	149	-----	-----
600	515	149	-----	-----
600	516	147	-----	-----
300	517	144	162	155
300	518	153	167	163
100	519	137	152	164
100	520	138	160	171

DISCUSSION AND CONCLUSION

In this screening study, mortality was noted for all animals which received a single oral dose of 600, 1000, or 2000 mg/kg of the test substance. One animal at the 600 mg/kg dose level displayed signs of loss of righting reflex and swollen face on the morning following dosing prior to death. The single-dose oral LD₅₀ was between 300 and 600 mg/kg. For animals receiving multiple daily doses, the two animals assigned to the 300 mg/kg/day dose level gained weight between Day 0 and Day 2 (18 and 14 grams), but lost weight between Day 2 and Day 4 (7 and 4 grams). One animal assigned to the 300 mg/kg/day dose level displayed signs of circling, head search, and retropulsion on the day following the fourth dose. All animals assigned to the 100 mg/kg/day dose level gained weight from the day of dosing to termination of the screen. The NOEL (No Observable Effect Level) for animals receiving repeated doses (four daily) under the conditions of the study was 100 mg/kg/day. No signs of dermal irritation were evident at the application site of the single female which received a topical exposure to the test substance.

REFERENCES

- Draize, J.H., Woodard, G., and Calvery, H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucus membranes, *J. Pharmacol. Exp. Ther.*, 82:377-390.

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