FINAL REPORT

CRUDE MCHM

HAEL No.: 97-0216 **EAN: 972790**
PM No.: 18717-00

ACUTE DERMAL TOXICITY STUDY IN THE RAT

GUIDELINE

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

AUTHOR

Lisa G. Bernard, M.S.

TESTING FACILITY

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

LABORATORY PROJECT ID

97-0216A1

STUDY SPONSOR

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

STUDY COMPLETION DATE

February 24, 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: 97-0216-1 STUDY DIRECTOR: BERNARD,L.G. PAGE 1
ACCESSION NUMBER: 972790 02/02/98

STUDY TYPE: ACUTE DERMAL TOXICITY

M. James 2/11/98
(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
08/19/97	PROTOCOL APPENDIX/AMENDMENT SUBMISSION	
08/21/97	CLINICAL SIGNS AT 48 HRS.	02/02/98
11/25/97	GROSS PATHOLOGY HISTOPATHOLOGY PATHOLOGY REPORT	11/25/97
02/02/98	FINAL REPORT REVIEW	02/02/98

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)].

Lisa Gayle Bernard

Lisa G. Bernard, M.S.
Study Director

2-24-98
Month/Day/Year

SIGNATURE PAGE

Lisa G. Bernard, M.S.
Study Director

2-24-98
Month/Day/Year

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

Feb 13, 1998
Month/Day/Year

John L. O'Donoghue, V.M.D., Ph.D. (Pathology)

2/17/98
Month/Day/Year

TABLE OF CONTENTS

	Page Number
ABSTRACT	6
STUDY AND TEST SUBSTANCE INFORMATION	8
Testing Facility	8
Project Participants	8
Sponsor	8
Test Substance Characterization	8
Study Dates	8
PURPOSE	9
MATERIALS AND METHODS	9
Test System	9
Husbandry	9
Experimental Design	10
Data Storage	11
Data Analysis	11
Protocol and Standard Operating Procedure Deviations	12
RESULTS	13
DISCUSSION	17
CONCLUSION	18
REFERENCES	18
APPENDIX	19

ABSTRACT

CRUDE MCHM

HAEL No.: 97-0216 EAN: 972790
PM No.: 18717-00

ACUTE DERMAL TOXICITY STUDY IN THE RAT

An acute dermal toxicity study was conducted in male and female rats administered a single limit dose of 2000 mg/kg of the test substance topically. The test substance, a clear and colorless liquid, was administered neat. One female rat was found dead the day after test substance application (Day 1) and a second female rat was found dead on Day 3.

For male rats, clinical signs observed during the 14-day observation period were limited to erythema (Days 1 to 4) and desquamation (Days 5 to 14) of the skin at the site of application for male rats For female rats, transient weakness (moderate to severe) was noted on the day following test substance application (Day 1). Prostration was noted on Day 2 for a single female rat which subsequently died. Stumbling, which was observed for four female rats on the day following test substance application, was either transient or observed prior to death. For female rats, abnormalities of the skin at the site of application were observed from Day 1 through study termination; erythema was observed on Days 1 and 2, desquamation was observed on Days 6 to 14, and induration was observed on Days 2 to 14. Additionally, lack of feces was observed on Day 2 and inguinal hair wet with urine was observed on Days 1 to 3 for the female rats. Red urine was noted for four female rats on Days 1, 2, or 3, therefore, the urine from all animals was tested for the presence of blood using a semi-quantitative dipstick (N-Multistix). The urine from rats with red discolored urine produced a positive response with the N-Multistix. The urine from rats which did not have red urine, produced a positive response with the N-Multistix for most of the rats on Day 1 and approximately half of the rats on Day 3. A positive N-Multistix result for animals which did not have red discolored urine was considered indicative of levels of blood in the urine too low to produce visible color changes. All animals which survived to scheduled necropsy gained weight during both weeks of the study.

The cause of death for rats which died after treatment with the test substance was not determined. Treatment-related gross or microscopic changes were observed only for female rats. For the two female rats which died, treatment-related gross lesions included distention of the urinary bladder with red urine, and/or hemorrhage in the glandular gastric mucosa. The lesions observed in the glandular gastric mucosa may have been due to consumption of the test substance during grooming or may have been due to stress. Darker than normal spleens were observed for the two female rats which had red urine and also died. Microscopic lesions consisted of atrophy and congestion of the splenic red pulp and/or atrophy and necrosis of the splenic white pulp. The white pulp atrophy may have been secondary to stress and the red pulp atrophy and congestion may have been related to stress and/or hemorrhage. However, splenic effects following dermal

application and wrapping are uncommon observations in this laboratory. In addition, splenic lesions have not been associated with wrapping (Parker and Gibson, 1995). Therefore, the splenic effects may be associated with test substance toxicity. Treatment-related lesions observed for one of the female rats that survived the 14-day observation period consisted of desquamation and minor induration of the skin at the application site grossly and consisted of focal necrosis and eschar formation on the skin at the application site microscopically.

The test substance was a dermal irritant as evidenced by focal necrosis and eschar formation on the skin at the application site. Based on the dermal LD_{50} calculated by combining male and female mortality data (>2000 mg/kg), the test substance was classified as 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:	Lisa G. Bernard, M.S.
Principal Investigator:	John W. Mosher, B.S.
Pathologist/Veterinarian:	Milan S. Vlaovic, D.V.M., Ph.D.

Sponsor

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

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

Test Substance Characterization

Test Substance Name:	Crude MCHM
HAEL No.:	97-0216
EAN:	972790
PM No.:	18717-00
SRID No.:	6-97
Physical State and Appearance:	Liquid, Clear and colorless
Source of Test Substance:	Eastman Chemical Company, Kingsport, TN
Laboratory Project ID:	97-0216A1

Study Dates

Study Initiation Date:	August 19, 1997
Experimental Start Date:	August 19, 1997
Experimental Completion Date:	November 26, 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. The male rats were 9 weeks of age and weighed 244 to 267 grams at the start of the study. The female rats were 11 weeks of age and weighed 224 to 237 grams at the start of the study. Rats were chosen for this study because they are a common representative species for toxicity studies. The rat is one of three 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 - 22°C and 51 - 61% 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 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.

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 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.

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 liquid test substance was administered as received.

Experimental Design, continued

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 substance in place for 24 hours. At the end of the exposure period, any residual test substance 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	541 - 545	546 - 550

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.

Data Analysis

No statistical procedures were required during the study.

Protocol and Standard Operating Procedure Deviations

There were no SOP or protocol deviations during the study.

RESULTS

Mortality

The number of animals dosed, the number of deaths at each dose level, and the day of death 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, 2	Days 1 and 3

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

For male rats, abnormal clinical signs were limited to erythema and desquamation of the skin at the site of application. For female rats, abnormal clinical signs consisted of moderate to severe weakness, prostration, red urine, inguinal hair wet with urine, lack of feces, stumbling, and erythema, desquamation, and induration of the skin at the site of application. The time of each observation and the number of animals involved at each dose level are listed in Table 3. Due to the observation of red urine, urine was tested for the presence of blood using a semi-quantitative dipstick product (N-Multistix, Miles Inc., Diagnostic Division, Elkhart, IN) on Days 1 and 3. The results of the N-Multistix tests are presented in Table 4.

TABLE 3

Table Of Clinical Observations

Dose (mg/kg)	Time	Clinical Signs	Number Of Animal Affected	
2000	Day 0	Appeared Clinically Normal	5/5 Males	5/5 Females
2000	Day 1 (during exposure)	Appeared Clinically Normal	5/5 Males	- - - -
		Moderate Weakness	- - - -	4/5 Females
		Severe Weakness	- - - -	1/5 Females
		Red Urine	- - - -	2/5 Females

Continued on the next page

TABLE 3, continued

Table Of Clinical Observations

Dose (mg/kg)	Time	Clinical Signs	Number Of Animal Affected	
2000	Day 1 (post-exposure)	Skin of Application Site: Erythema	5/5 Males	5/5 Females
		Moderate Weakness	----	2/5 Females
		Severe Weakness	----	3/5 Females
		Red Urine	----	3/5 Females
		Inguinal Hair Wet With Urine	----	1/5 Females
		Stumbling	----	4/5 Females
		Found Dead	----	1/5 Females
2000	Day 2	Skin of Application Site: Erythema	5/5 Males	4/4 Females
		Skin of Application Site: Induration	----	3/4 Females
		Severe Weakness	----	1/4 Females
		Prostration	----	1/4 Females
		Red Urine	----	2/4 Females
		Inguinal Hair Wet With Urine	----	3/4 Females
		Lack of Feces	----	3/4 Females
2000	Day 3	Found Dead	----	1/4 Females
		Skin of Application Site: Erythema	5/5 Males	----
		Skin of Application Site: Induration	----	3/3 Females
		Red Urine	----	1/3 Females
		Inguinal Hair Wet With Urine	----	1/3 Females
2000	Day 4	Skin of Application Site: Erythema	5/5 Males	----
		Skin of Application Site: Induration	----	3/3 Females
2000	Day 5	Skin of Application Site: Desquamation	5/5 Males	----
		Skin of Application Site: Induration	----	3/3 Females
2000	Day 6	Skin of Application Site: Desquamation	5/5 Males	2/3 Females
		Skin of Application Site: Induration	----	3/3 Females
2000	Day 7	Skin of Application Site: Desquamation	5/5 Males	3/3 Females
		Skin of Application Site: Induration	----	3/3 Females
2000	Days 8-10	Appeared Clinically Normal	2/5 Males	----
		Skin of Application Site: Desquamation	3/5 Males	3/3 Females
		Skin of Application Site: Induration	----	2/3 Females
2000	Days 11-13	Appeared Clinically Normal	4/5 Males	1/3 Females
		Skin of Application Site: Desquamation	1/5 Males	2/3 Females
		Skin of Application Site: Induration	----	2/3 Females
2000	Day 14	Appeared Clinically Normal	3/5 Males	1/3 Females
		Skin of Application Site: Desquamation	2/5 Males	2/3 Females
		Skin of Application Site: Induration	----	2/3 Females

TABLE 4

N-Multistix Results For Animals With Red Urine

Dose (mg/kg)	Time	Clinical Signs	Number Of Animals Affected	
2000	Day 1	Large (+++) Amount	-----	3/3 Females
2000	Day 3	Moderate Amount (non-hemolyzed)	-----	1/1 Females

N-Multistix Results For Animals Which Did Not Have Red Urine

Dose (mg/kg)	Time	Clinical Signs	Number Of Animals Affected	
2000	Day 1	Negative	1/5 Males	-----
		Small (+) Amount	1/5 Males	-----
		Moderate (++) Amount	3/5 Males	2/2 Females
		Large (+++) Amount	-----	-----
2000	Day 3	Negative	3/5 Males	-----
		Trace Amount (non-hemolyzed)	-----	1/2 Female
		Small (+) Amount	1/5 Males	-----
		Moderate (++) Amount	1/5 Males	-----
		Large (+++) Amount	-----	1/2 Female

Body Weights

All animals which survived to study termination gained weight during both weeks of the study. 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 7	Day 14
MALE RATS				
2000	541	244	251	268
2000	542	264	274	297
2000	543	267	286	305
2000	544	258	264	285
2000	545	265	271	298
FEMALE RATS				
2000	546	229	246	260
2000	547	237	Died on Day 3	204[β]
2000	548	231	238	254
2000	549	224	234	237
2000	550	230	Died on Day 1	[α]

[α] A terminal body weight was not recorded for any animal which died within 24 hours of dosing.
[β] Terminal body weight recorded at necropsy.

Pathology Findings

For male rats, no treatment-related gross lesions were observed at necropsy. For two female rats which died, treatment-related changes observed at necropsy consisted of minor distention of the urinary bladder with red urine (1/2) and darker than normal spleens (2/2). In addition, minimal hemorrhage in the glandular gastric mucosa was observed for one female rat which died; this gross lesion was not considered treatment-related by the pathologist. For the three female rats that survived the 14-day observation period, treatment-related lesions consisted of minor desquamation (1/3) and minor induration (1/3) of the skin at the application site.

For male rats, microscopic examination of gross lesions observed in the liver, pancreas, spleen, stomach, thymus, skin, lungs, and kidneys revealed no treatment-related changes. For the two female rats which died, treatment-related microscopic changes consisted of focal necrosis (1/1) and minimal hemorrhage (1/1) in the glandular gastric mucosa, moderate or severe atrophy (2/2) and moderate or severe congestion (2/2) of the splenic red pulp, and/or minor atrophy (2/2) and a minimal necrosis (1/2) of the splenic white pulp. For one female rats that survived the 14-day observation period, treatment-related minimal focal necrosis (1/1) and eschar formation (1/1) on the skin at the application site was observed.

A detailed record of the incidence and severity of all gross and microscopic findings is presented in computer-generated tables which are included in the Appendix.

DISCUSSION

In the dermal toxicity study, male and female rats were administered a single limit dose of 2000 mg/kg of the test substance topically. Mortality was 0% for male and 40% for female rats. The acute dermal LD_{50} for this test substance was greater than 2000 mg/kg for both male and female rats.

Clinical signs observed during the 14-day observation period for male rats were limited to erythema and desquamation of the skin at the site of application. Female rats were slightly more sensitive to this test substance than male rats, exhibiting moderate to severe weakness, prostration, stumbling, red urine, inguinal hair wet with urine, lack of feces, and erythema, desquamation, and induration of the skin at the site of application. Transient weakness (moderate to severe) was noted in female rats on the day following test substance application (Day 1). Prostration was noted on Day 2 for a single female rat which subsequently died. Stumbling was observed for four female rats on Day 1; two of these animals subsequently died. Abnormalities of the skin at the site of application were observed from Day 1 through study termination; erythema was observed for male rats on Days 1 to 4 and for female rats on Days 1 and 2, induration was observed for female rats on Days 2 to 14, and desquamation was observed for male rats on Days 5 to 14 and for female rats on Days 6 to 14. All animals which survived to scheduled necropsy gained weight during both weeks of the study.

Since red urine was noted for some female rats, the urine from all rats was tested for the presence of blood using N-Multistix dipsticks. For rats which had red urine, the N-Multistix results were +3 on Day 1 and moderate amount (non-hemolyzed) on Day 3. For rats which did not have red urine, N-Multistix results ranged from +1 to +2 for six of seven rats on Day 1 and ranged from trace amount (non-hemolyzed) to +3 for four of seven rats on Day 3. A positive N-Multistix response for animals which did not have red discolored urine was considered indicative of levels of blood in the urine too low to produce visible color changes.

The cause of death for rats which died after treatment with the test substance was not determined. Treatment-related gross or microscopic changes were observed only for female rats. For the two female rats which died, treatment-related gross lesions included distention of the urinary bladder with red urine and darker than normal spleens. In addition, hemorrhage in the glandular gastric mucosa was observed for one of these female rats. Although the gross stomach lesion was not considered treatment-related by the pathologist, the microscopic appearance of the gastric mucosa suggest that the lesions observed were associated with exposure to the test substance, therefore it seems likely that the gross lesion was also treatment-related. The lesions observed in the glandular gastric mucosa may have been due to consumption of the test substance during grooming or may have been due to stress.

Splenic lesions were observed for the two female rats which had red urine and also died. The lesions consisted of atrophy and congestion of the splenic red pulp and/or atrophy and focal necrosis of the splenic white pulp. The pathologist attributed the white pulp atrophy to stress and

the red pulp atrophy and congestion to stress and/or hemorrhage. Splenic effects following dermal application and wrapping are uncommon observations in this laboratory. In addition, splenic lesions have not been associated with wrapping (Parker and Gibson, 1995). Therefore, these splenic effects may be associated with test substance toxicity.

Treatment-related lesions observed for one of the female rats that survived the 14-day observation period consisted of desquamation and minor induration of the skin at the application site grossly and consisted of focal necrosis and eschar formation on the skin at the application site microscopically.

CONCLUSION

The test substance was a dermal irritant as evidenced by focal necrosis and eschar formation on the skin at the application site. Based on the dermal LD_{50} calculated by combining male and female mortality data (>2000 mg/kg), the test substance was classified as 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.

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.

Parker, G.A. and Gibson, W.B. (1995). Liver lesions in rats associated with wrapping of the Torso. *Toxicol. Path.*, **23**, 507-512.

APPENDIX

HAEL No. 97-0216
EAN 972790

PATHOLOGY REPORT

Test Substance: Crude MCHM

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.

RESULTS

GROSS PATHOLOGY:

Male Rats - 2000 mg/kg exposure group: No exposure-related changes were observed.

All rats survived the observation period.

Incidental findings included minor edema (1/5) and minimal pallor (1/5) of the glandular gastric mucosa, minimal thymus hemorrhage (2/5), minimal granular appearance of the liver (3/5), minimal red discoloration (3/5) and firmness of the pancreas (3/5), and minimal or moderate pallor (2/5) and minor reduction in the size (1/5) of the spleen.

Female Rats - 2000 mg/kg exposure group: Exposure-related changes included minor distention of the urinary bladder with red urine (1/5), darker than normal spleens (2/5), and minor desquamation (1/5) and minor induration (1/5) of the skin at the application site.

Single rats died on Days 1 and 3, and the remaining three rats survived the observation period.

Incidental findings included the lungs that did not collapse completely when the thoracic cavity was opened during necropsy (1/5), minimal or moderate thymus hemorrhage (2/2), minor edema (2/5) and minimal hemorrhage (1/5) in the glandular gastric mucosa, moderate pallor of the liver (1/5), darker than normal kidneys (1/5), wet inguinal hair by urine (1/5), brown discolored inguinal hair by urine (1/5), and minimal or minor hydrometra (3/5).

HISTOPATHOLOGY:

Selected gross lesions were processed for microscopic evaluation.

Male Rats - 2000 mg/kg exposure group: No exposure-related changes were observed. Incidental findings included minimal congestion (1/3), a minimal chronic focal inflammation (1/3), and minor cytoplasmic vacuolization of the hepatocytes (3/3) in the liver; minimal chronic focal inflammation (2/3) of the splenic capsule, and minimal thymus hemorrhage (2/2).

Freedom_0000960_0020

Female Rats - 2000 mg/kg exposure group: Exposure-related changes included minimal focal necrosis (1/1) and minimal hemorrhage (1/1) in the glandular gastric mucosa, moderate or severe atrophy (2/2) and moderate or severe congestion (2/2) of the splenic red pulp, minor atrophy (2/2) and a minimal necrosis (1/2) of the splenic white pulp, and minimal focal necrosis (1/1) and eschar formation (1/1) on the skin at the application site.

Incidental findings included minimal or minor hydrometra (3/3), moderate congestion of the liver (1/2), minimal or moderate cytoplasmic vacuolization of the hepatocytes (2/2), minimal or moderate thymus hemorrhage (2/2), minimal congestion (1/1), a minimal chronic focal inflammation of the alveolar wall in the lungs (1/1), minor congestion of the kidneys (1/1), and a minimal mineralizations in the renal tubules (1/1).

COMMENTS:

No concurrent control group was available for observation. Therefore, the conclusions in this study were based on the experience of the pathologist with control animals from other studies.

Microscopic lesions which may be associated with the exposure were found in the spleens of Rats 547 and 550 and skin of Rat 548.

Splenic lesions included atrophy and congestion of the red pulp, and atrophy and focal necrosis of the white pulp. A typical lesion was characterized by depletion of the splenic hematopoietic tissue that was replaced by mature red blood cells (congestion), and by lymphocyte depletion and necrosis in the periarteriolar lymphoid sheaths and marginal zones. Lymphocyte depletion in the periarteriolar lymphoid sheaths and marginal zones of the spleen was probably secondary to stress. It has been reported that stress, mediated by steroid release from the adrenal cortex, is associated with lymphoid tissue involution in the spleen (Zbinden, 1963). Atrophy and congestion observed in the hematopoietic tissue of the spleen was also considered to be related to stress and/or hemorrhage.

The skin of Rat 548 showed a focal full thickness necrosis of the epidermis with scab formation. This type of lesion is consistent with strong skin irritation.

Incidental microscopic findings were observed in the liver, spleen, thymus, kidneys, lungs, and uterus.

Freedom_0000960_0021

The liver changes included congestion, chronic focal inflammation, and cytoplasmic vacuolization of hepatocytes. A minimal congestion of the liver was observed in Rat 541. This change was considered secondary to incomplete bleeding prior to necropsy. Minimal chronic focal inflammation of the liver was present in Rat 544. The foci of chronic inflammation were routinely distributed throughout the liver and were characterized by small accumulations of predominantly mononuclear cells.

Cytoplasmic vacuolizations in hepatocytes were present in Rats 541, 543, 544, 547, and 550. On gross observation, cytoplasmic vacuolization in hepatocytes was characterized as pallor of the liver. Cytoplasmic vacuolizations in hepatocytes was characterized by the presence of small membrane-bound vacuoles. These vacuoles may have contained either glycogen or lipids; however, the unambiguous determination of vacuolar content depends on avoiding embedment procedures that extract glycogen and lipid and on the use of special stains.

Incidental splenic lesions consisted of minimal chronic focal inflammations of the splenic capsule. This change is characterized by thickening of the capsule due to accumulation of fibroblasts, lymphocytes, and macrophages. The origin of this lesion could not be identified; however, inflammatory lesions of the splenic capsule usually originate from adjacent abdominal organs.

Thymic hemorrhage was an incidental finding observed in Rats 543, 544, 549, and 550. Thymic hemorrhage was considered an agonal lesion, although it may have also occurred as a result of dissection of the thymus during necropsy.

The kidneys of Rat 550 showed minimal tubular mineralizations. This lesion was characterized by tiny lamellated concretions or microliths located in the lumen of the renal tubules. The microliths were not associated with evidence of cell degeneration. Tubular mineralizations are commonly observed in untreated control rats, and were not considered exposure-related.

Minimal chronic focal inflammations of the alveolar wall was present in the lungs of Rat 550. This lesion is occasionally observed in untreated-control rats, and was not considered exposure-related.

Congestion of the liver, lungs, and kidneys of Rat 550 were considered agonal phenomena not related to the exposure.

Minimal or minor hydrometra was observed in rats 546, 547, and 550. Hydrometra is the dilation of the uterus with an accumulation of intraluminal fluid during the estrus cycle of the rat.

Freedom_0000960_0022

CONCLUSIONS

Exposure to 2000 mg/kg of the test substance over clipped bare skin for 24 hours produced necrosis of the skin with scab formation.

REFERENCES:

Zbinden, G.: Experimental and clinical aspects of drug toxicity. In: Garattini, S. and Shore, P. A. (eds.): Advances in Pharmacology, Academic Press, New York, 1963, pp. 1-112.

11/26/97
Milan S. Vlaovic, D.V.M., Ph.D.

11/26/97
Reviewed by
John L. O'Donoghue, V.M.D., Ph.D.

MSV:sji
11/24/97

Freedom_0000960_0023

SUMMARY GROSS PATHOLOGY INCIDENCE TABLE - MALE RATS

	2000 MG/KG
TRACHEA	5
LUNGS	5
THYMUS	5
HEMORRHAGE	2
HEART	5
ESOPHAGUS	5
STOMACH	5
STOMACH, GLANDULAR	
EDEMA	1
PALLOR	1
DUODENUM	5
JEJUNUM	5
ILEUM	5
CECUM	5
COLON	5
RECTUM	5
LIVER	5
HEPATIC CAPSULE	
GRANULAR APPEARANCE	3
KIDNEYS	5
URINARY BLADDER	5
PITUITARY GLAND	5
ADRENALS	5
PANCREAS, NOS	5
DISCOLORATION,RED	3
THYROID GLANDS	5
PARATHYROID GLANDS	5
SPLEEN	5
PALLOR	2
SMALL	1
MESENTERIC LYMPH NODES	5
BONE MARROW	5
BRAIN	5
EYES	5
SALIVARY GLANDS	5
ADIPOSE TISSUE	5
SKIN, NOS	5
HAIR	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

	2000 MG/KG
TRACHEA	5
LUNGS	5
COLLAPSE INCOMPLETE ON THORACOTOMY	1
THYMUS	5
HEMORRHAGE	2
HEART	5
ESOPHAGUS	5
STOMACH	5
STOMACH, GLANDULAR	
EDEMA	2
HEMORRHAGE	1
DUODENUM	5
JEJUNUM	5
ILEUM	5
CECUM	5
COLON	5
RECTUM	5
LIVER	5
PALLOR	1
KIDNEYS	5
COLOR-DARKER THAN NORMAL	1
URINARY BLADDER	5
DISTENTION	1
PITUITARY GLAND	5
ADRENALS	5
PANCREAS, NOS	5
THYROID GLANDS	5
PARATHYROID GLANDS	5
SPLEEN	5
COLOR-DARKER THAN NORMAL	2
MESENTERIC LYMPH NODES	5
BONE MARROW	5
BRAIN	5
EYES	5
SALIVARY GLANDS	5
ADIPOSE TISSUE	5
SKIN, NOS	5
SKIN OF BACK	
SCLEROSIS/INDURATION	1
DESQUAMATION	1
HAIR	5
HAIR OF INGUINAL REGION	
HAIRCOAT, WET BY URINE	1
DISCOLORATION,BROWN	1
FALLOPIAN TUBES	5
VAGINA	5
UTERUS	5
HYDROMETRA	3
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 #	541	542	543	544	545
DAYS ON TEST	14	14	14	14	14
TRACHEA	X	X	X	X	X
LUNGS	X	X	X	X	X
THYMUS	X	X			X
HEMORRHAGE			1	1	
HEART	X	X	X	X	X
ESOPHAGUS	X	X	X	X	X
STOMACH	X		X	X	X
STOMACH, GLANDULAR					
EDEMA		2			
PALLOR		1			
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
HEPATIC CAPSULE					
GRANULAR APPEARANCE	1		1	1	
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
DISCOLORATION,RED	1	1	1		
THYROID GLANDS	X	X	X	X	X
PARATHYROID GLANDS	X	X	X	X	X
SPLEEN		X	X		X
PALLOR	3			1	
SMALL	2				
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	X	X	X	X	X
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, X-NORMAL BUT NOT COLLECTED, 1-MINIMAL, 2-MINOR, 3-MODERATE, 4-SEVERE, P-PRESENT, A-ABSENT, *-SEE COMMENT REPORT

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE - FEMALE RATS

2000 MG/KG

ANIMAL #	546	547	548	549	550
DAYS ON TEST	14	3	14	14	1
TRACHEA	X	X	X	X	X
LUNGS	X	X	X	X	
COLLAPSE INCOMPLETE ON THORACOTOMY					P
THYMUS	X	X	X		
HEMORRHAGE				1	3
HEART	X	X	X	X	X
ESOPHAGUS	X	X	X	X	X
STOMACH	X		X	X	
STOMACH, GLANDULAR					
EDEMA		2			2
HEMORRHAGE		1			
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
PALLOR		3			
KIDNEYS	X	X	X	X	
COLOR-DARKER THAN NORMAL					2
*URINARY BLADDER	X	X	X	X	
DISTENTION					2
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	
COLOR-DARKER THAN NORMAL		4			3
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
SKIN OF BACK					
SCLEROSIS/INDURATION			2		
DESQUAMATION			2		
*HAIR	X		X	X	
HAIR OF INGUINAL REGION					
HAIRCOAT, WET BY URINE					2
DISCOLORATION,BROWN		3			
FALLOPIAN TUBES	X	X	X	X	X
VAGINA	X	X	X	X	X
UTERUS			X	X	
HYDROMETRA	2	1			2

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

INDIVIDUAL ANIMAL GROSS PATHOLOGY INCIDENCE TABLE - FEMALE RATS

	2000 MG/KG				
ANIMAL #	546	547	548	549	550
DAYS ON TEST	14	3	14	14	1
OVARIES	X	X	X	X	X
CERVIX UTERI	X	X	X	X	X

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

GROSS PATHOLOGY COMMENT REPORT

DAY	DOSE LEVEL	ANIMAL #	COMMENT
16	2000 MG/KG	550	URINARY BLADDER WAS DISTENDED WITH RED URINE.
16	2000 MG/KG	547	INGUINAL HAIR WAS STAINED BROWN BY URINE.
16	2000 MG/KG	543	PANCREAS WAS FIRM (2).
16	2000 MG/KG	541	PANCREAS WAS FIRM (2).
16	2000 MG/KG	542	PANCREAS WAS FIRM (2).

SUMMARY HISTOPATHOLOGY INCIDENCE TABLE - MALE RATS

	2000 MG/KG
LIVER	3
CONGESTION	1
INFLAMMATION, CHRONIC FOCAL	1
HEPATOCYTE	
CYTOPLASMIC VACUOLIZATION	3
PANCREAS, NOS	3
SPLEEN	2
SPLENIC CAPSULE	
INFLAMMATION, CHRONIC FOCAL	2
STOMACH	1
THYMUS	2
HEMORRHAGE	2
SKIN, NOS	0
LUNGS	0
KIDNEYS	0

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

SUMMARY HISTOPATHOLOGY INCIDENCE TABLE - FEMALE RATS

	2000 MG/KG
UTERUS	3
HYDROMETRA	3
STOMACH	2
STOMACH, GLANDULAR	
NECROSIS, FOCAL	1
HEMORRHAGE	1
LIVER	2
CONGESTION	1
HEPATOCYTE	
CYTOPLASMIC VACUOLIZATION	2
SPLEEN	2
SPLENIC RED PULP	
ATROPHY	2
CONGESTION	2
SPLENIC LYMPHATIC FOLLICLE	
ATROPHY	2
NECROSIS, FOCAL	1
SKIN, NOS	1
SKIN, APPLICATION SITE	
NECROSIS, FOCAL	1
ESCHAR	1
THYMUS	2
HEMORRHAGE	2
LUNGS	1
CONGESTION	1
ALVEOLAR WALL	
INFLAMMATION, CHRONIC FOCAL	1
KIDNEYS	1
CONGESTION	1
RENAL TUBULE	
MINERALIZATION	1

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

INDIVIDUAL ANIMAL HISTOPATHOLOGY INCIDENCE TABLE - MALE RATS

	2000 MG/KG				
ANIMAL #	541	542	543	544	545
DAYS ON TEST	14	14	14	14	14
LIVER					
CONGESTION	1				
INFLAMMATION, CHRONIC FOCAL				1	
HEPATOCYTE					
CYTOPLASMIC VACUOLIZATION	2		2	2	
PANCREAS, NOS	N	N	N		
SPLEEN					
SPLENIC CAPSULE					
INFLAMMATION, CHRONIC FOCAL	1			1	
STOMACH		N			
THYMUS					
HEMORRHAGE			1	1	
SKIN, NOS					
LUNGS					
KIDNEYS					

KEY: P-PRESENT, A-ABSENT, N-NORMAL ,1-MINIMAL, 2-MINOR, 3-MODERATE, 4-SEVERE, *-SEE COMMENT REPORT

INDIVIDUAL ANIMAL HISTOPATHOLOGY INCIDENCE TABLE - FEMALE RATS

2000 MG/KG

ANIMAL #	546	547	548	549	550
DAYS ON TEST	14	3	14	14	1
UTERUS					
HYDROMETRA	2	1			2
STOMACH					N
STOMACH, GLANDULAR					
NECROSIS, FOCAL		1			
HEMORRHAGE		1			
LIVER					
CONGESTION					3
HEPATOCYTE					
CYTOPLASMIC VACUOLIZATION		3			1
SPLEEN					
SPLENIC RED PULP					
ATROPHY		4			3
CONGESTION		4			3
SPLENIC LYMPHATIC FOLLICLE					
ATROPHY		2			2
NECROSIS, FOCAL					1
SKIN, NOS					
SKIN, APPLICATION SITE					
NECROSIS, FOCAL			1		
ESCHAR			P		
THYMUS					
HEMORRHAGE				1	3
LUNGS					
CONGESTION					1
ALVEOLAR WALL					
INFLAMMATION, CHRONIC FOCAL					1
KIDNEYS					
CONGESTION					2
RENAL TUBULE					
MINERALIZATION					1

KEY: P-PRESENT, A-ABSENT, N-NORMAL ,1-MINIMAL, 2-MINOR, 3-MODERATE, 4-SEVERE, *-SEE COMMENT REPORT