



NIPA HARDWICKE INC.

3411 SILVERSIDE RD., 104 HAGLEY BLDG., WILMINGTON, DE 19810
TELEPHONE (302) 478-1522 • FAX (302) 478-4097

May 2, 1997

Ms. Debbie Lumpkins
Office of OTC Drug Evaluation (HFD 560)
Food and Drug Administration
9201 Corporate Boulevard
Room 560
Rockville, MD 20850

Re: Docket 75N-183H
Chloroxylonol (PCMX)
Draft Protocol - 13 Week Dermal Range-Finding Study In Mice

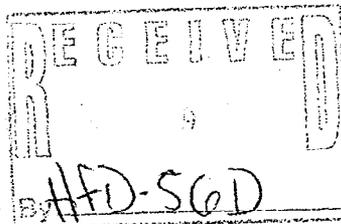
Dear Ms. Lumpkins:

NIPA Hardwicke Inc. is currently performing a 14 day MTD study based on the U.S. EPA principles outlined in Debra L. Bowen's letter to us of May 6, 1996. Once this evaluation is completed, we will make an appointment to discuss with you the results obtained.

Prior to meeting with you to present the developed 14 day MTD data, we are submitting our draft protocol for a 13 Week Dermal Range-Finding Study In Mice. This 91 day evaluation will be the basis for your requested 24 month evaluation. The only difference from this draft protocol will be the use of the "high dose" determined in the 14 day MTD evaluation.

Sincerely,

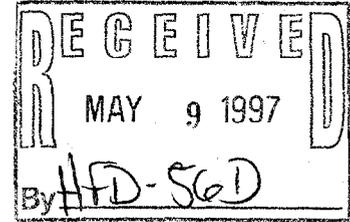
Irving Gottlieb
Director of Sales and Marketing, Biocides



cc: G. Kramzar
Dr. W. Guess

75N-183H

PR 6



**DRAFT
PROTOCOL**

A 13-Week Dermal Range-Finding Study of PCMX in Mice

Study No.: WIL-_____

For

NIPA Laboratories
3411 Silverside Road
Wilmington, DE 19180

By

WIL Research Laboratories, Inc.
Ashland, Ohio 44805-9281

April 11, 1994

A 13-WEEK DERMAL RANGE-FINDING STUDY OF PCMX IN MICE

WIL Study No.: WIL- _____

Sponsor Project No.: _____

I. OBJECTIVE OF STUDY

The objective of this study is to evaluate the possible toxic effects of the test article when administered dermally to mice for 91 days (minimum) and to provide information of setting the doses for a chronic oncogenicity study.

The study will be conducted in compliance with the FDA Good Laboratory Practice Standards, 22 CFR Part 58.

II. PERSONNEL INVOLVED IN THE STUDY**A. Sponsor Representative**

Mr. Gary Kramzar
Vice President

B. WIL Study Director

Christopher P. Chengelis, Ph.D., D.A.B.T.
Associate Director of Toxicology

C. WIL Toxicology Department Responsibilities

1. E. Crosby Tompkins, Ph.D., D.A.B.T.
Vice President, Director of Toxicology
2. Dennis J. Naas, B.S.
Assistant Director of Toxicology
3. Gary R. Kiplinger, B.S.
Associate Toxicologist
4. Kerin Clevidence, B.S.
Acting Section Head I - Pathology and
Developmental Toxicology Laboratory
5. Lisa Simon, B.S., M.T. (ASCP)
Supervisor of Clinical Pathology
6. Lynn Myers, A.S.
Section Head I - Histology
7. Stanley E. Kopp
Systems Manager

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A 13-Week Dermal Range-Finding Study in Mice

II. PERSONNEL INVOLVED IN THE STUDY (continued)

8. Sally Keets, A.S.
Manager of In-Life Facilities
9. Deborah L. Little
Manager of Quality Assurance
10. Ian C. Lamb, Ph.D.
Manager of Pharmacy
11. Kevin Oberholtzer, B.S.
Manager of Technical Report Writing
12. Loren W. Severs, M.S.
Manager of Analytical Chemistry
13. Robert Dahlgren, D.V.M., Ph.D., Diplomate A.C.V.P.
Director of Pathology

III. STUDY SCHEDULE DATA

- A. Proposed Experimental Start Date: To be determined.
- B. Proposed Necropsy Date: To be determined.
- C. Proposed Draft Report Date: To be determined.

IV. TEST MATERIAL DATA

- A. Identification: PCMX (Chloroxylenol)
- B. Lot Number: To be provided by the Sponsor.
- C. Purity: Test article purity data are the responsibility of the Sponsor.
- D. Stability: Test article stability data are to be provided by the Sponsor.
- E. Physical Description: To be documented by WIL Research.
- F. Storage Conditions: Sealed container at room temperature.

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A 13-Week Dermal Range-Finding Study in Mice

IV. TEST MATERIAL DATA (continued)

- G. Reserve Samples: A 1 gram sample will be taken from each batch of test material and stored in the Archives at WIL Research Laboratories, Inc.
- I. Personnel Safety Data: A dust respirator, safety glasses, appropriate protective clothing and latex gloves will be worn when preparing or handling the solutions and neat test article. A material safety data sheet or equivalent will be requested from the client.

V. TEST SYSTEM

- A. Species: Mouse
- B. Strain: Charles River CD[®]-1 (ICR)BR
- C. Source: The Charles River Breeding Laboratories, Inc.
9801 Shaver Road
Portage, Michigan 49081
- D. Number of Animals: Approximately 60 males and 60 females will be purchased.
- E. Approximate Age: Animals will be approximately 4 weeks of age when received, and no more than 6 weeks of age at study initiation.
- F. Identification System: Animals will be identified by tattoo. Individual cage cards will be affixed to each cage and will display the animal number, group number and study number.
- G. Justification for Selection: This species and strain of animal is recognized to be appropriate for subchronic dermal toxicity and oncogenicity studies. The CD[®]-1 mouse will be utilized because it is a widely used strain for which significant historical control data are available.

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A 13-Week Dermal Range-Finding Study in Mice

VI. SPECIFIC MAINTENANCE SCHEDULE**A. Animal Housing**

Weanling animals will be housed 3 per cage by sex in clean suspended wire-mesh cages for approximately 3 days following receipt. Thereafter, all animals will be housed individually in suspended wire-mesh cages in an environmentally controlled room. The cages will be elevated above cage-board or other suitable material. Animals will be housed in general accordance with DHEW Publication No (NIH), 85-23 (revised 1985).

B. Environmental Conditions

Controls will be set to maintain room temperature at $72^{\circ} \pm 3^{\circ}\text{F}$ and relative humidity at approximately 30-70%. Fluorescent lighting will provide illumination for 12 hours per day. Temperature and relative humidity will be recorded once daily.

C. Drinking Water

Tap water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to WIL Standard Operating Procedures. Municipal water supplying the laboratory will be analyzed for contaminants according to WIL Standard Operating Procedures to ascertain that none are present in concentrations that would be expected to affect the outcome of the study.

D. Diet

Purina[®] Certified Rodent Chow #5002 will be offered *ad libitum* during the study. Analyses of the certified feed for the presence of heavy metals and pesticides will be provided by the manufacturer to ensure that none are present in concentrations that would be expected to affect the outcome of the study.

VII. EXPERIMENTAL DESIGN**A. Animal Receipt and Quarantine**

Each animal will be inspected by a qualified technician upon receipt. Mice judged to be in good health will be placed immediately in quarantine for approximately seven to ten days. All animals will be weighed and assigned a permanent animal number. During the acclimation period, each animal will be observed twice daily for changes in general appearance and behavior. All animals will receive a detailed physical examination at the time of animal selection for randomization.

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A 13-Week Dermal Range-Finding Study in Mice

VII. EXPERIMENTAL DESIGN (continued)

The animals will be allowed a pretest week during which body weights, food consumption and general health will be monitored but they will not receive the test material.

B. Randomization

At the conclusion of the acclimation period, animals judged to be suitable for testing will be assigned to groups at random using a computer-generated program. At this time, the animal numbers and corresponding body weights will be entered into WIL's Computer Data Management System. A printout containing the animal numbers and individual exposure group assignments will be generated based on body weight stratification into a block design. Animals will then be arranged into the groups according to the printout. Body weights at randomization will be within $\pm 20\%$ of the mean for each sex. If after randomization significant differences between groups exist, new randomizations will be generated until group mean body weights are homogeneous.

C. Route and Rationale of Test Article Administration

The route of administration will be topical (dermal) since the intended use of the test article indicates that this will be the major route of exposure of human exposure.

D. Organization of Test Groups, Exposure Levels and Treatment Regimen1. Organization of Test Groups

The following diagram presents the study group arrangement.

<u>Group Number</u>	<u>Test Material</u>	<u>Dosage Concentration</u>	<u>Number of Animals</u>	
			<u>Males</u>	<u>Females</u>
1 (optional)	None	0	10	10
2	Acetone	0	10	10
3	PCMX	TBD	10	10
4	PCMX	TBD	10	10
5	PCMX	TBD	10	10

TBD = To be determined

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A 13-Week Dermal Range-Finding Study in Mice

VII. EXPERIMENTAL DESIGN (continued)2. Treatment Regimen

The animals will be dosed once per day, seven days per week for 13 weeks (minimum).

3. Method of Administration

The hair will be clipped from the intrascapular region of back of each animal. This will be done the day before initiation and as often as needed thereafter (at least one time each week).

The test article at the rate of 1 ml/kg will be applied. Animals in the control groups will be maintained and observed in the same manner as the test group animals but will not be dosed with test article. The vehicle control group animals will be dosed with acetone at a volume equal to that used for administration of test article.

The appropriate amount of test article will be applied evenly by gentle slow dispensation (allowing the solution to spread in a slow even circular pattern without running) from a calibrated positive displacement pipeter. The site will be dried with a gentle stream of unheated air as appropriate. Sites will not be dressed or wrapped. Sites will be washed (with acetone and gauze swabs) to remove residual build-up once per week.

4. Adjustment of Dosages

Dosages will be adjusted weekly based on the most current individual body weights.

VIII. PARAMETERS TO BE EVALUATEDA. Viability Observations

All animals will be observed for mortality/moribundity twice daily, once in the morning and once in the afternoon. Moribund animals will be euthanized to ensure that tissues will not be lost due to autolysis.

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VIII. PARAMETERS TO BE EVALUATED (continued)B. Clinical Observations

A clinical examination will be performed daily for all animals approximately 3 to 4 hours post-dosing. Observations will include but are not limited to changes in the skin, fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems function, somatomotor activity and behavior patterns. Findings noted at the clinical examinations (positive or negative) will be recorded for individual animals.

C. Dermal Observations

Application sites will be examined for erythema, edema and other dermal findings once per week. Erythema and edema will be evaluated in accordance with the method of Draize (Appendix A) based on the four-step grading system of very slight, slight, moderate and severe. Other dermal findings, if present, will be noted.

E. Individual Body Weights

Individual body weights will be recorded weekly, beginning one week prior to test material administration.

F. Individual Food Consumption

Individual food consumption will be recorded weekly, beginning one week prior to test material administration.

G. Clinical Pathology (optional)

The following clinical pathologic parameters will be evaluated on all surviving animals just at study termination. These animals will be fasted overnight before taking blood samples. The blood samples will be collected at the time of necropsy from the vena cava. Half the mice (per sex per group) will be used for hematology and the remaining mice for clinical chemistry.

1. Hematology

Hemoglobin	Reticulocyte counts (if signs of anemia are present) ^a
Hematocrit	
Erythrocyte count	MCH
Total leukocyte count	MCV
Differential leukocyte count ^a	MCHC
Platelet count	

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VIII. EXPERIMENTAL OBSERVATIONS (continued)

a = To be done initially only on control and high dose groups. If a potential effect is noted, this evaluation may be extended to the low and mid dose groups.

2. Blood Chemistry

Serum alanine aminotransferase	Total bilirubin
Serum aspartate aminotransferase	Total protein
Serum alkaline phosphatase	Albumin
Glucose	Globulin
Blood urea nitrogen	A/G ratio
Total cholesterol	
Creatine kinase	

I. Anatomic Pathology1. Macroscopic Examination

A complete necropsy will be conducted on all animals dying spontaneously, euthanized *in extremis* or at study termination. Animals euthanized *in extremis* or at study termination will be anesthetized with carbon dioxide and exsanguinated. Necropsy will include examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities including viscera. At the time of necropsy the following tissues and organs will be collected and placed in 10% neutral buffered formalin:

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VIII. EXPERIMENTAL OBSERVATIONS (continued)

Adrenals (2)	Ovaries with oviducts (2)
Aorta	Pancreas
Bone with marrow (sternum)	Pituitary
Brain	Prostate
Eyes with optic nerve (2)	Seminal vesicles (2)
Gastrointestinal tract	Skin (treated and untreated)
Stomach	Spleen
Duodenum	Testes with epididymides (2)
Jejunum	Thymus
Ileum	Thyroid gland (both lobes with parathyroids, if present)
Heart	Urinary bladder
Kidneys (2)	All gross lesions
Liver (sections of two lobes)	
Lungs (including bronchi, fixed by inflation with fixative)	
Lymph node (mesenteric)	

2. Organs Weights (optional)

The following organs from all animals at the final necropsy will be weighed.
(Pair organs will be weighed together.):

Brain
Kidneys
Liver
Ovaries
Testes

Organ to body weight ratios will be calculated.

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VIII. EXPERIMENTAL OBSERVATIONS (continued)3. Microscopic Examination

Microscopic examination of hematoxylin - eosin stained paraffin sections will be performed on the tissues listed in protocol section VIII.I.1. for all animals in the naive control and high dose groups and from all mice that die or are euthanized *in extremis*. The lungs, liver, kidneys, treated and untreated skin, and gross lesions will be examined from all other animals. If a potential target organ is noted in the high dose group, that tissue will also be examined in the vehicle control, low and mid dose groups.

IX. STATISTICAL METHODS

All analyses will be two-tailed for significance levels of 5% and 1%. All means will be presented with standard deviations. All statistical test will be performed by a Digital® Equipment Corporation computer with appropriate programming. Analysis of weekly body weights, body weight changes and food consumption as well as clinical pathology values (excluding differential white cell counts other than segmented neutrophils and lymphocytes), and absolute and relative organ weights will be analyzed by a one-way analysis of variance comparison of the control group to each treated group by Dunnett's test(1).

Dermal score data will be inspected by the study director and an appropriate method of analysis chosen, documented in the raw data, and described in the final report.

X. QUALITY ASSURANCE

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance Good Laboratory Practice regulations, adherence to the protocol and to WIL Standard Operating Procedures. The final report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor to assure that the final report accurately describes the conduct and the findings of the study.

This is a regulated study and will be included on the master schedule.

XI. RECORDS TO BE MAINTAINED

All original raw data records will be stored in the Archives at WIL Research Laboratories, Inc. Records to be retained will include, but are not limited to, the following:

- A. Protocol and protocol amendments
- B. Master protocol computer printout
- C. WIL study personnel involved in the conduct of the study

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XI. RECORDS TO BE MAINTAINED (continued)

- D. Study schedule
- E. Purina® Certified feed lot records
- F. Animal receipt and identification records including purchase orders and shipping records
- G. Laboratory animal inventory
- H. Quarantine body weights and observation records
- I. Documentation of animal selection for study
- J. Test material preparation records with Balance accuracy records
- K. Computer randomization records
- L. Body weight (weekly) computer archive reports
- M. Food consumption (weekly) computer archive reports
- N. Clinical observation computer archive reports
- O. Clinical pathology computer archive reports
- P. Room temperature and humidity records
- Q. Animal room cleaning records
- R. Mortality/Moribundity records
- S. Computer raw data edit records
- T. Unscheduled deaths/euthanization records
- U. Gross pathological and histopathological raw data computer records

XII. WORK PRODUCT

Sponsor will have title to all documentation records, raw data, specimens or other work products generated during the performance of the study. Within six months following submission of the final report all work products (including tissues, blocks and slides) will be transferred to the Sponsor. In the event the Sponsor cannot accommodate transfer of all or part of the work product such materials will be retained in the archives at WIL Research Laboratories indefinitely as directed by the Sponsor. WIL Research Laboratories will charge a standard annual storage fee for the retention of such work product. Raw data in magnetic form will be retained by WIL Research Laboratories in compliance with regulatory requirements.

XIII. REPORTS

The report will contain all information necessary to conform with current FDA and OECD specifications.

The contents of the report will be as follows:

A. Text

1. Summary
2. Introduction

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XIII. REPORTS (continued)

3. Objective
4. Experimental Procedures
5. Results of Clinical Observations
6. Results of Dermal Observations
7. Results of Body Weights
8. Results of Food Consumption
9. Results of Clinical Pathology Evaluation
10. Results of Macroscopic Examination
11. Results of Organ Weights
12. Results of Microscopic Examination
13. Discussion and Conclusion

B. Tables

1. Summary of Clinical Observations
2. Summary of Dermal Observations
3. Summary of Mean Weekly Body Weights and Body Weight Changes
4. Summary of Mean Food Consumption (g/animal/day)
5. Summary of Clinical Pathologic Values
6. Summary of Macroscopic Findings
7. Summary of Organ Weight Values
8. Summary of Organ/Body Weight Values
9. Summary of Microscopic Findings
10. Individual Dermal Observations
11. Individual Weekly Body Weights
12. Individual Food Consumption (g/animal/day)
13. Individual Clinical Pathology Values
14. Individual Macroscopic Findings
15. Individual Organ Weight Values
16. Individual Organ/Body Weight Values
17. Individual Microscopic Findings

C. Final Report: Three copies will be supplied

XIV. PROTOCOL MODIFICATION

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and Sponsor Representative.

FILE 94.99**A 13-Week Dermal Range-Finding Study in Mice****XV. ANIMAL WELFARE ACT COMPLIANCE**

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

1. The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.
2. Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures.
3. Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.
4. Methods of euthanasia used during this study are in conformance with the above referenced regulation.

XVI. PROTOCOL APPROVAL

NIPA Laboratories
3411 Silverside Rd.
Wilmington, DE 19810

WIL Research Laboratories, Inc.
Ashland, Ohio 44805-9281

Mr. Gary Kramzar
Vice President

Christopher P. Chengelis, Ph.D., D.A.B.T.
Study Director

Date

Date

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References

1. **BMDP-79 Biomedical Computer Programs, University of California Press, Berkeley, California, 1979.**

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APPENDIX A

SCORING CRITERIA FOR DERMAL REACTIONSEvaluation of Dermal Reactions*

<u>Value</u>	<u>Erythema and Eschar Formation</u>	<u>Computer Designation</u>
0	No erythema	No erythema
1	Very slight erythema (barely perceptible, edges of area not well defined)	Very slight erythema
2	Slight erythema (pale red in color and edges definable)	Slight erythema
3	Moderate to severe erythema (definite red in color and area well defined)	Moderate erythema
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)	Severe erythema

	<u>Edema Formation</u>	<u>Computer Designation</u>
0	No edema	No edema
1	Very slight edema (barely perceptible, edges of area not well defined)	Very slight edema
2	Slight edema (edges of area well defined by definite raising)	Slight edema
3	Moderate edema (raised approximately 1 mm)	Moderate edema
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)	Severe edema

*Draize, J. H., 1965. The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Assoc. of Food and Drug Officials of the U.S., Topeka, Kansas.