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 **Clariant**



September 13, 2002

Document Control Officer  
Dockets Management Branch (HFA-305)  
**FOOD and DRUG ADMINISTRATION**  
5630 Fishers Lane, Room 1061  
Rockville, MD 20852

RE: Docket No. 75N183H  
Topical Antimicrobial Drug Products for Over-the-Counter Use:  
Tentative Final Monograph for Health-Care Antiseptic Drug Products  
**Chloroxylenol (PCMX) – 13 Week Dermal Study & 24 Month Dermal Draft Protocol**

Dear Sirs:

Enclosed are two copies each of the following for Agency review:

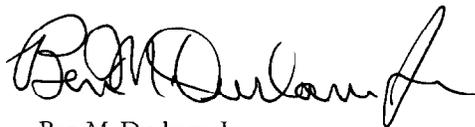
**Completed Testing- 13 Week Dermal Toxicity Study of PCMX in Mice**  
**Draft Protocol- 24 Month Dermal Carcinogenicity Study of PCMX in Mice**

The Dermal Toxicity Study was performed in accordance with the Agency's review of and comments on the previously submitted draft protocol. This study is being submitted under the mandates set forth by the Agency to meet the requirements for classification of Chloroxylenol (PCMX) as a Category I Active Ingredient for Topical Handwash Applications.

A Draft Protocol for the 24 Month Dermal Carcinogenicity Study of PCMX in Mice is enclosed as part of this submission to the Agency for the Agency's review and approval. This protocol is based on the results of the 13 Week Dermal Toxicity Study also enclosed.

We request a meeting with the Agency's reviewers and other appropriate personnel to discuss both submissions. Please advise a meeting date that is convenient for all Agency personnel involved.

Sincerely;

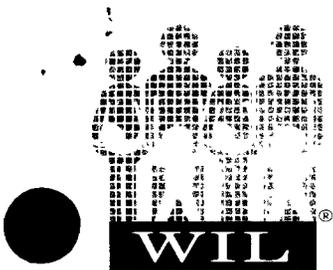


Ben M. Durham, Jr.  
Product Safety Manager  
Functional Chemicals Division

cc: D. Andrews  
I. Gottlieb

**75N-183H**

**PR 9**



## PROTOCOL

### A 24-MONTH DERMAL CARCINOGENICITY STUDY OF PCMX IN MICE

#### Submitted To:

**Clariant Corporation** C  
625 East Catawba Ave.  
Mount Holly, NC 28120

  
9/12/2002

**WIL Research Laboratories, Inc.**  
1407 George Road  
Ashland, OH 44805-9281

- I. Objective:** The objective of this study is to evaluate the potential carcinogenic effects of the test article when administered dermally to mice daily for 24 months.

This protocol has been designed and the study will be conducted in general accordance with the United States Food and Drug Administration (FDA), Japanese Ministry of Health, Labour and Welfare (MHLW) and the International Conference on Harmonization (ICH).

The study will be conducted in compliance with the U.S. FDA Good Laboratory Practice Standards, 21 CFR Part 58.

**II. Personnel Involved in the Study:**

**A. Sponsor Representative:**

Irving Gottlieb  
27 Jarombek Drive  
Towaco, NJ 07082  
Tel: (973) 334-9227  
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E-mail: irv.gottlieb@clariant.com

**B. Sponsor Monitor:**

Wallace R. Guess  
Consultant in Dermato-Toxicology  
408 County Road 102  
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**C. WIL Study Director:**

Jay F. Harriman, Ph.D.  
Staff Toxicologist  
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**D. WIL Deputy Director:**

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

**E. WIL Departmental Responsibilities:**

Jozef J.W.M. Mertens, Ph.D., D.A.B.T.  
Associate Director, General Toxicology

Teresa D. Morris, B.S.  
Operations Manager, Toxicology

Sally A. Keets, A.S.  
Acting Senior Operations Manager, Vivarium

Ronald E. Wilson, B.S.  
Director, Informational Systems

Daniel W. Sved, Ph.D.  
Director, Metabolism and Analytical Chemistry

Theresa M. Rafeld  
Group Supervisor, Formulations Laboratory

Susan C. Haley, B.S.  
Group Supervisor, Clinical Pathology

Carol A. Kopp, B.S.  
Manager, Gross Pathology and  
Developmental Toxicology Laboratory

John T. Yarrington, D.V.M., Ph.D., D.A.C.V.P.  
Senior Pathologist

Michael Safron, A.S., H.T. (A.S.C.P.)  
Manager, Histology

Barbara L. Smith, D.V.M., M.S., Ph.D., D.A.C.V.S.  
Director, Laboratory Animal Resources

Philip L. Stetson, M.D., Ph.D.  
Associate Director, Analytical Chemistry



Lewis E. Kaufman, M.S.  
Senior Operations Manager, Study Analysis and Reports

Deborah L. Little, B.S., RQAP-GLP  
Manager II, Quality Assurance

David A. Wilkie, D.V.M., M.S., D.A.C.V.O.  
The Ohio State University, Consulting Ophthalmologist

**III. Study Schedule:**

Proposed Animal Receipt Date:	To be determined.
Proposed Experimental Start Date:	To be determined.
Proposed Necropsy Date:	To be determined.
Proposed Audited Report Date:	To be determined.

**IV. Test Article Information:**

**A. Test Article:**

- 1. Identification:** PCMX (Chloroxylenol)  
(also known as Nipacide®)
- 2. Supplier:** Sponsor
- 3. Batch Number:** To be provided by Sponsor.
- 4. Purity:** Assumed to be 100% (based upon information provided by the Sponsor).
- 5. Stability:** Stability data are the responsibility of the Sponsor.
- 6. Physical Description:** To be documented by WIL Research Laboratories, Inc.
- 7. Storage Conditions:** Room temperature, protected from light.
- 8. Reserve Samples:** Retention samples will be collected and stored in accordance with Standard Operating Procedures.



**9. Personnel Safety:**

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 sponsor.

**10. Vehicle:**

Test article will be dissolved in acetone. The solvent will be purchased by WIL Research Laboratories, Inc. Lot number and supplier will be documented in the raw data.

**V. Test System:****A. Species:**

Mouse

**B. Strain:**

Charles River CrI:CD-1<sup>®</sup>(ICR)BR

**C. Source:**

Charles River Laboratories, Inc.  
(Facility to be documented in the raw data)

**D. Number of Animals:**

280 males and 280 females will be purchased, 240/sex will be assigned to the toxicology groups. Animals not utilized on study will be returned to stock colony or euthanized by CO<sub>2</sub> inhalation and discarded.

**E. Approximate Age and Body Weight Range:**

Animals will be approximately 4 weeks of age when received, and approximately 6 weeks of age at initiation of dosing. Animals are expected to weigh approximately 20 to 30 grams at initiation of dosing.

**F. Identification System:**

Animals will be identified uniquely by tail tattoo displaying the animal number. 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 as appropriate for carcinogenicity studies. The CD-1<sup>®</sup> mouse will be utilized

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because it is a widely used strain for which significant historical data are available and was the species and strain used for the preliminary studies on PCMX conducted at WIL Research Laboratories, Inc.

**VI. Specific Maintenance Schedule:**

**A. Animal Housing:**

Upon arrival, weanling animals will be housed 3 per cage by sex in clean, suspended, wire-mesh cages for at least 3 days following receipt. Thereafter, all animals will be housed individually in clean, suspended, stainless steel wire-mesh cages in an environmentally controlled room. The cages will be elevated above cage-board or other suitable material. The facilities at WIL Research Laboratories, Inc. are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

After the start of dosing, the position of each cage bank will be rotated to a different location in the room on a monthly basis.

**B. Environmental Conditions:**

The room assigned will be equipped with an anteroom to be used for preparation and gowning, and access will be limited. Individuals entering the room will be expected to follow precautions specified by WIL SOPs.

Controls will be set to maintain an average daily temperature of  $71 \pm 5^{\circ}\text{F}$  ( $22 \pm 3^{\circ}\text{C}$ ) and an average daily relative humidity of  $50 \pm 20\%$ . Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. Temporary adjustments to the light/dark cycles may be made to accommodate protocol-specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

**C. Drinking Water:**

Reverse osmosis-treated water will be available *ad libitum*. Water treatment equipment and the automatic watering system will be serviced regularly. Municipal water supplying the laboratory will be analyzed for contaminants according to WIL Standard Operating Procedures to ascertain that none are

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present in concentrations that would be expected to affect the outcome of the study.

**D. Diet:**

PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 (meal) will be offered *ad libitum* during the study. Each lot utilized will be identified and recorded. Standard operating procedures provide specifications for acceptable levels of heavy metals and pesticides that are reasonably expected to be present in the diet without interfering with the purpose or conduct of the study. Each lot of feed has been analyzed to assure specifications are met. Feeders will be changed and sanitized once per week.

**VII. Experimental Design:**

**A. Animal Receipt and Acclimation:**

Each animal will be inspected by qualified personnel upon receipt. Animals judged to be in good health will be allowed an acclimation period of approximately 14 days. Animals will receive a permanent animal number upon individual housing. All animals at the time of individual housing will have body weights taken, food consumption determination initiated and detailed physicals performed. During the acclimation period, each animal will be observed twice daily for changes in general appearance and behavior. The animals will be allowed a pretreatment week (as part of the acclimation period) during which body weights and food consumption will be determined and general health will be monitored, but they will not receive the test article. All animals will receive a detailed physical examination approximately one week prior to the initiation of dosing and at the time of animal selection for randomization.

**B. Randomization:**

Near the end of the acclimation period, animals judged to be suitable for testing will be assigned to groups at random using a computer program. At this time, the animal numbers will be entered into the WIL Toxicology Data Management System (WTDMS™). A printout containing the animal numbers and individual 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.

**C. Route and Rationale of Test Article Administration:**

The route of administration will be dermal since the intended use of the test article indicated that dermal contact is the route of exposure for humans.

**D. Organization of Test Groups, Dosage Levels and Treatment Regimen:****1. Organization of Test Groups:**

The following table presents the study group arrangement:

Group Number	Treatment	Approximate* Dosage (mg/kg/day)	Dose Concentration (%)	Dose Volume ( $\mu$ l)	Number of Animals	
					Males	Females
1	Acetone (vehicle control)	0	0	50	60	60
2	PCMX	50	3	50	60	60
3	PCMX	167	10	50	60	60
4	PCMX	500	30	50	60	60

\* - Based on a 30 gram mouse.

**2. Justification of Dose Selection:**

Dose selection is based on the results of a 13-week study of dermally administered PCMX in mice (WIL-304003). In this study, 60% PCMX in acetone resulted in an increased incidence of granulocytic hyperplasia of the bone marrow, thought to correlate to increased demand for circulating leukocytes in conjunction with dermal inflammation. The 30% concentration of PCMX resulted in dermal findings similar to those of the 60% concentration, but did not reveal secondary systemic changes. Dermal findings at both levels included thickening and scabbing of the skin, epidermal hyperplasia and hyperkeratosis. The 30% dose of PCMX is thus the approximate Maximum Tolerated Dose for dermal administration of PCMX in acetone and is the high dose chosen for the carcinogenicity study. The 3% dose should be a No-Observed-Effect-Level (NOEL) based on previously conducted short term dermal studies of PCMX. The mid-dose level is spaced in an attempt to produce a gradation of toxic effects.

**3. Treatment Regimen:**

The vehicle and test article formulations will be applied seven days per week for 24 months (until the day before the scheduled necropsy). Test article will be applied at a constant volume of 50  $\mu$ l per mouse. Vehicle control group will be dosed with acetone.

#### **4. Administration:**

The hair will be clipped from the back of each animal, from the scapula (shoulder) to the wing of the ileum (hipbone) and halfway down each flank on each side of the animal. This will be done the day before initiation of dosing and as often as needed thereafter (at least one time each week).

The test article solutions will be drawn into an appropriate size calibrated pipette. The test article will be slowly and evenly discharged from the pipette onto the target dose area.

The test and control articles will be applied to a single site on each animal. The area covered by test article will be measured and recorded once per week for one representative (close to the mean body weight) animal of each sex in each group. In addition, the corners of the application site will be marked with indelible ink to allow proper identification of the treated and untreated skin. The application sites will not be dressed or wrapped.

The test sites will be washed with acetone and gauze swabs once per week prior to dosing.

#### **E. Duration of Study:**

The first day of dosing is day 0, and the first week of dosing is week 0. The duration of the study will be 24 months. However, after 18 months the duration of the study (or portions thereof) may be reduced if survival is reduced to the following points. The high dose group may be terminated before 24 months if survival reaches 20 animals without terminating the other groups. If the number of survivors in any group appears to be approaching 20 per group, the Sponsor will be contacted to discuss possible termination of a group or the entire study. Males and females will be considered separately.

#### **F. Test Article Preparation and Analysis:**

##### **1. Test Article Preparation:**

The test article will be dissolved in vehicle on a weight-to-volume basis using routine methods. Test article preparations will be prepared weekly and subdivided into daily aliquots.



## 2. Stability and Homogeneity of the Test Article Formulations:

Stability of the test article will be conducted before initiation of the study. Solutions of test article of sufficient volume at each of the selected dosage levels will be prepared. Two samples will be taken from the middle of all concentrations and analyzed. Duplicate samples from the middle portion of each preparation will be stored under refrigerated conditions for 7 and 14 days and will be analyzed for stability.

## 3. Analysis of Test Article Concentrations:

Throughout the study, samples of each dosing formulation (test article and vehicle) will be collected and frozen. Samples will be analyzed for test article concentration (prior to use) weekly for the first four weeks, at week 12 and approximately every 13 weeks thereafter. In addition, frozen samples of each formulation will be retained at WIL Research until issuance of the final report. All chemical analyses on dosing solutions will be performed by WIL Research. A description of analytical methods and the analytical results will be included in the study report as an appendix.

## 4. Analyses of Neat Test Article:

The neat PCMX will be analyzed for purity and stability at 6-month intervals during the study, and at study termination.

### VIII. Parameters to be Evaluated:

#### A. Viability Observations (All Groups):

All animals will be observed for mortality/moribundity each morning and afternoon. Moribund animals will be euthanized so that tissues will not be lost due to autolysis.

#### B. Clinical Observations:

##### 1. Daily Observations:

A clinical examination will be performed once daily approximately three hours after 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 examination will be recorded for individual animals; the

condition of the animals without signs will be documented as a general comment.

## **2. Dermal Observations:**

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

## **3. Detailed Physical Examination:**

All animals will receive a detailed physical examination weekly, beginning during acclimation upon individual housing of the animals. 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. This examination will also include recording of the time of onset (date), appearance, size, location and growth of palpable masses. The absence or presence of findings will be recorded for individual animals.

### **C. Individual Body Weights:**

Individual body weights will be recorded at least once weekly, beginning during acclimation upon individual housing of the animals, through 13 weeks and every four weeks thereafter.

### **D. Individual Food Consumption:**

Individual food consumption will be recorded at least weekly, beginning during acclimation upon individual housing, through 13 weeks, and every four weeks thereafter.

### **E. Ophthalmic Examination:**

A board-certified veterinary ophthalmologist, using an indirect ophthalmoscope (or other equivalent suitable equipment) will examine all animals. An appropriate mydriatic agent will be administered prior to the examination. The examinations will be performed before initiation of dosing and at week 52.

## G. Clinical Pathology:

Total and differential WBC counts will be performed for all animals euthanized *in extremis* and for all animals at the time of scheduled necropsy. Animals will be euthanized by CO<sub>2</sub> asphyxiation for necropsy and blood collection. Blood will be collected from the vena cava. (The anticoagulant will be EDTA.)

## H. Anatomic Pathology:

### 1. Macroscopic Examination:

A complete necropsy will be conducted on all animals. Mice will be euthanized by CO<sub>2</sub> asphyxiation. A necropsy will be conducted on animals dying spontaneously, euthanized *in extremis*, or at scheduled necropsies. At 24 months, all surviving animals will be euthanized and necropsies will be performed. Necropsies 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 (except where noted):

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Adrenal glands (2)	Lymph node (mesenteric)
Aorta	Mammary gland (females only)
Bone with marrow	Nasal turbinates <sup>d</sup>
Sternum	Ovaries with oviducts(2)
Femur	Pancreas
Bone marrow smear <sup>a</sup>	Parathyroids <sup>e</sup>
Brain	Peripheral nerve (sciatic)
Cerebrum Level 1	Pituitary
Cerebrum Level 2	Prostate
Cerebellum with pons medulla	Salivary glands [mandibular (2)]
Epididymides (2) <sup>c</sup>	Seminal vesicles (2)
Eyes with optic nerves (2) <sup>b</sup>	Skeletal muscle (rectus femoris)
Gallbladder	Skin
Gastrointestinal tract	Treated <sup>f</sup>
Esophagus	Untreated
Stomach	Spinal Cord
Duodenum	Cervical
Jejunum	Midthoracic
Ileum	Lumbar
Cecum	Spleen
Colon	Testes (2) <sup>c</sup>
Rectum	Thymus
Harderian glands (2)	Thyroid
Heart	Tongue
Kidneys (2)	Trachea
Liver (sections of two lobes)	Urinary bladder
Lungs (including bronchi, fixed by inflation with fixative)	Uterus with cervix
	Vagina
	All gross lesions (when possible)

<sup>a</sup> = Not taken from animals found dead, not placed in formalin; to be examined only if scientifically warranted.

<sup>b</sup> = To be placed in Davidson's Solution.

<sup>c</sup> = To be placed in Bouin's Solution.

<sup>d</sup> = Sections I and III of nasal turbinates according to the method of Young.<sup>1</sup>

<sup>e</sup> = Parathyroids will be examined microscopically if in the plane of section and in all cases where a gross lesion is present.

<sup>f</sup> = The entire treated area will be collected. Three sections will be prepared for microscopic examination.

## 2. Organ Weights:

The following organs from animals euthanized at the scheduled necropsy will be weighed (from first 10 mice/sex/group)

Adrenals	Ovaries with oviducts
Brain	Spleen
Epididymides	Testes
Heart	Thyroid with parathyroids*
Kidneys	Thymus
Liver	Uterus

\*Weighed after fixation.

Paired organs will be weighed together. Organ-to-body-weight and organ-to-brain-weight ratios will be calculated.

## 3. Microscopic Examination:

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues mentioned in Section VIII.H.1. for all animals euthanized *in extremis* or that die spontaneously, and for all animals in the control and high dose groups. In addition, all gross lesions and tissue masses from all animals will be examined. Remaining tissues from Groups 2 and 3 will be retained for possible future examination. Microscopic examination may be extended to other organs, tissues or groups at an additional cost if a potential target organ is noted based on histopathological examination of tissues from the high dose group or on changes in other parameters. Special stains may be used at the discretion of the pathologist to further characterize lesions and changes. Any special stains used will be documented in the individual animal's data and interpretation of results will be included in the final report.

## IX. Statistical Methods:

The main objectives of the statistical analysis are:

- To assess the effect of the test article on survival.
- To assess the effect of the test article on tumor incidence.

For the purpose of the statistical analysis, the test article treated groups will be compared solely with the control group (Group 1).

Data collected from males and females will be analyzed separately.

Statistical tests and comparisons of each in-life and mortality variable will be assessed at the overall 0.05 level of significance. The Bonferroni inequality will be used to control the false-positive error rate at the overall 0.05 level when several pairwise comparisons are made (i.e., each of the k comparisons for a given variable will be conducted at the 0.05/k level of significance). Unless specified otherwise, all tests and comparisons will be conducted as two-sided.

#### **A. In-Life Data:**

Body weight, body weight changes and food consumption, absolute and relative organ weights as well as clinical pathology values (excluding differential white cell counts other than lymphocytes and neutrophils), will be subjected to a one-way analysis of variance (ANOVA).<sup>2</sup> Pairwise comparisons will be made between test article treated groups and the control group by Dunnett's Test<sup>3</sup> if the ANOVA is statistically significant ( $p < 0.05$ ).

#### **B. Mortality Data:**

The Kaplan-Meier<sup>4</sup> survival curve will be calculated for each sex/treatment group, and displayed graphically over time. The generalized Wilcoxon test for survival will be used to compare the homogeneity of survival rates across the groups at the 0.05 significance level. If the survival rates are significantly different, the generalized Wilcoxon test<sup>5</sup> will be used to make pairwise comparisons of each treated group with the control group. The pairwise comparisons will be made using Bonferroni adjusted p-values at the 0.05 significance level.

#### **C. Tumor Incidence Data:**

Electronic tumor data set will be generated from the raw data in accordance with FDA guidance<sup>6</sup> and will be utilized for statistical assessment of tumor incidence.

The incidence of tumors will be analyzed by Peto's mortality-prevalence method,<sup>7</sup> without continuity correction, incorporating the context (incidental or fatal) in which tumors are observed. The following fixed intervals will be used for incidental tumor analyses: weeks 0-52, 53-78, 79-92, 93-end of study, and terminal sacrifice. Palpable tumors that are detected prior to necropsy will be analyzed with Peto's mortality-independent method incorporating the day of detection.

Tumor data will be analyzed by tumor type and by organ/tissue system. Tumors of similar histologic origin may be combined, at the discretion of the study director. For each tumor type a 1-sided trend test will be conducted using the dose coefficients. In addition, pairwise comparisons with the control group will be conducted for each active treatment group.

An exact permutation test will be conducted for analyses with low tumor incidence. Statistical significance will be determined according to the following guidelines: trend tests will be conducted at the 0.025 and 0.005 significance levels for common and rare tumors, respectively.<sup>8,9,10,11</sup> Pairwise comparisons with the control group will be conducted at the 0.01 and 0.05 significance levels for common and rare tumors, respectively.<sup>12</sup> A rare tumor will be defined as one in which the historical spontaneous tumor rate is less than 1%.

**X. Quality Assurance:**

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with the study protocol and protocol amendments, WIL standard operating procedures and the appropriate provisions of FDA (21 CFR Part 58). The raw data and draft 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 study will be included on the WIL master list of regulated studies.

**XI. Records to be Maintained:**

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored in the Archives at WIL Research Laboratories, Inc. as described in Section XII.

**XII. Work Product:**

The Sponsor will have title to all documentation records, raw data, specimens and other work product generated during the performance of the study. All work product, including: raw paper data, pertinent electronic storage media and specimens, will be retained at for five years in the Archives at WIL Research Laboratories, Inc. following issuance of the final report. Thereafter, WIL Research Laboratories will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.



Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, Inc. to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, Inc. will not be responsible for shipment following delivery to the common carrier.

**XIII. Reports:**

The draft and final reports will contain a summary, test article data, methods and procedures, a copy of the protocol and amendments (if any), appropriate individual animal and summary data tables and an interpretation and discussion of the study results. The reports will be comprehensive and shall define level(s) inducing toxic effects, as well as "no-effect" level(s) under the condition of this investigation. The reports will contain all information necessary to conform with current FDA, MHLW, and ICH specifications.

WIL Research Laboratories, Inc. will provide one (1) copy of an Audited Draft Report upon completion of the study prior to issuance of the Final Report. One (1) revision is included as part of the cost of the study, from which Sponsor's reasonable revisions and suggestions will be incorporated into the Final Report as appropriate. Additional changes or revisions may result in additional fees. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two (2) month time frame following submission. WIL will submit the Final Report within one (1) month following receipt of comments. Two (2) copies of the Final Report (PDF) will be provided; requests for additional copies of the Final Report may result in additional charges. An electronic tumor data set will also be provided.

**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.

**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:



- The Sponsor's 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.
- 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.
- 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.
- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

#### **XVI. References:**

1. Young, J.T. (1981) Histopathologic Examination of the Rat Nasal Cavity. *Fundamental and Applied Toxicology*, 1:309-312.
2. Snedecor, G.W. and Cochran, W.G. (1980) One way classifications; analysis of variance. In: *Statistical Methods, Seventh Edition*. Iowa State University Press, Ames, IA, pp. 215-237.
3. Dunnett, C.W. (1964) New tables for multiple comparisons with a control. *Biometrics*, 20:482-491.
4. Kaplan E.L. and Meier P. (1958) Non-parametric estimation from incomplete observations. *J. American Stat. Assoc.*, 53: 457-481.
5. Gehan, E.A. (1965), "A Generalized Wilcoxon Test for Comparing K Samples Subject to Unequal Patterns of Censorship," *Biometrika*, 52, 203-223.
6. FDA (1999), "Guidance for Industry, Providing Regulatory Submissions in Electronic Formats – NDAs," Center for Drug Evaluation and Research.
7. Peto, R., M.C. Pike, N.E. Day, R.G. Gray, P.N. Lee, S. Parish, J. Peto, S. Richards, and J. Wahrendorf (1980), "Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-term Animal

Experiments," in Long-term and Short-term Screening Assays for Carcinogens: An Critical Appraisal, World Health Organization.

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**XVII. Protocol Approval:**

Sponsor approval received via \_\_\_\_\_ on \_\_\_\_\_  
Date

**Clariant Corporation**

\_\_\_\_\_  
Irving Gottlieb  
Sponsor Representative

\_\_\_\_\_  
Date

**WIL Research Laboratories, Inc.**

\_\_\_\_\_  
Jay F. Harriman, Ph.D.  
Study Director

\_\_\_\_\_  
Date

\_\_\_\_\_  
Christopher P. Chengelis, Ph.D., D.A.B.T.  
Director, Toxicology

\_\_\_\_\_  
Date



## 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

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<u>Value</u>	<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.