FINAL REPORT

Thirteen-Week Oral (Gavage) Toxicity of Mesozeaxanthin in Han Wistar Rats with a 4-Week Recovery

Test Article:
Mesozeaxanthin

Sponsor:
Howard Foundation Holdings Limited
Whitehill House
Granhams Road
Great Shelford
Cambridge, CB2 5JY
United Kingdom

Testing Facility:
Gene Logic Laboratories Inc. (Gene Logic)
610 Professional Drive
Gaithersburg, MD 20879

Gene Logic Study Number:
1567-04370

Author:
C.J. George Chang, DVM, MS, PhD, DABT

Study Completion Date:
October 10, 2006

Page 1 of initial pages only – full report can be obtained at:
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COMPLIANCE STATEMENT

Thirteen-Week Oral (Gavage) Toxicity of Mesozeaxanthin in Han Wistar Rats with a 4-Week Recovery

This study was conducted in compliance with the U.S. FDA Good Laboratory Practice (GLP) Regulations for Non-clinical Laboratory Studies (21 CFR Part 58), OECD Good Laboratory Practice Principals (GLP) [ENV/MC/CHEM(98)17], and Japanese MHLW Good Laboratory Practice (GLP) Standards (Ordinance No. 21).

- The test article formulation stability analysis, the dose verification analysis, and analytical chemistry methods performed for the Sponsor by Industrial Organica were not conducted in compliance with the above regulatory guidelines.

- Stability analysis was not performed on lot 5 09 J1 EPZ of the formulated test article.

- There were a few instances where initials and/or date were not recorded in a timely fashion. Senior study personnel subsequently reviewed data; data were not compromised by the lack of timely documentation.

- Data entry of 'No Visible Lesions' for the Lacrimal Gland was not entered at the time of necropsy for termination and recovery rats.

- The proposed experimental completion date was not included in the protocol (OECD 8.2.3).

There were no deviations from the aforementioned regulations that affected the quality or integrity of the study or the interpretations of the results in this report.

Study Director:

C.J. George Chang, DVM, MS, PhD, DABT

Date: 10/10/06
QUALITY ASSURANCE STATEMENT

Thirteen-Week Oral (Gavage) Toxicity of Mesozeaxanthin in Han Wistar Rats with a 4-Week Recovery

This study, 1567-04370 entitled “Thirteen-Week Oral (Gavage) Toxicity of Mesozeaxanthin in Han Wistar Rats with a 4-Week Recovery” was inspected/audited by Quality Assurance in accordance with Gene Logic’s Standard Operating Procedures, the protocol, FDA, OECD, and MHLW Good Laboratory Practice Regulations. All findings were reported to the Study Director and Testing Facility Management.

<table>
<thead>
<tr>
<th>Type of Audit</th>
<th>Date Audited</th>
<th>Study Director</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol</td>
<td>April 13, 2005</td>
<td>April 13, 2005</td>
<td>April 13, 2005</td>
</tr>
<tr>
<td>SD 8 Dose Administration</td>
<td>April 28, 2005</td>
<td>May 3, 2005</td>
<td>May 3, 2005</td>
</tr>
<tr>
<td>SD 21 Dose Administration</td>
<td>May 11, 2005</td>
<td>May 18, 2005</td>
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</tr>
<tr>
<td>Ophthalmology Exams</td>
<td>July 18, 2005</td>
<td>July 21, 2005</td>
<td>July 21, 2005</td>
</tr>
<tr>
<td>Necropsy</td>
<td>July 20, 2005</td>
<td>July 25, 2005</td>
<td>July 25, 2005</td>
</tr>
<tr>
<td>Raw Data/Draft Final Report</td>
<td>December 18-5-8, 2005</td>
<td>December 8, 2005</td>
<td>December 8, 2005</td>
</tr>
<tr>
<td>Histopathology Raw Data</td>
<td>December 12, 2005</td>
<td>December 13, 2005</td>
<td>December 13, 2005</td>
</tr>
</tbody>
</table>

Action has been taken in response to all items listed by Quality Assurance. It is concluded that the final report accurately reflects Gene Logic’s Standard Operating Procedures and the raw data for this study.

William C. Spare, MS
Sr. Manager Quality Assurance
SIGNATURE PAGE
Thirteen-Week Oral (Gavage) Toxicity of Mesozeaxanthin in Han Wistar Rats with a 4-Week Recovery

Author:

[Signature]

C.J. George Chang, DVM, MS, PhD, DABT
Study Director

10/10/06
Date

Peer Review:

[Signature]

C. Steven Godin, PhD, DABT
Senior Director, Toxicology

11/9/06
Date
SUMMARY

Thirteen-Week Oral (Gavage) Toxicity of Mesozeaxanthin in Han Wistar Rats with a 4-Week Recovery

The purpose of this study was to determine the potential toxicity of Mesozeaxanthin when administered once daily to male and female Han Wistar rats for 13 consecutive weeks, and to assess in a 4-week recovery period the delayed onset of any toxicity or persistence or reversibility of any effects noted earlier during the 13-week dosing phase. Han Wistar rats (100 total; 50/sex) were randomly assigned to one of four groups (10-15 sex/group) and administered with Corn Oil (control) or Mesozeaxanthin at dose levels of 2, 20, or 200 mg/kg/day (Groups 2-4, respectively) for 13 consecutive weeks by oral gavage. Parameters evaluated included mortality, clinical observations, body weights, ophthalmology, clinical pathology, organ weights, gross pathology, and histopathology.

No compound-related mortality, clinical signs of toxicity, changes in body weights, ophthalmology, clinical pathology, gross pathology, or histopathology were noted.

Based on the results of this study, the no-observed-adverse-effect-level (NOAEL) of Mesozeaxanthin in rats is >200 mg/kg/day when administered orally for 13 consecutive weeks.
STUDY PERSONNEL AND TEST SITES

Study Director: C.J. George Chang, DVM, MS, PhD, DABT
Alternate Study Director: C. Steven Godin, PhD, DABT (From June 14 to July 5, 2005)
Toxicology Associate: Karl Fraser, MS
Report Associate: Rachna Arora, MS
Head Technicians: Michael Johnson (April 12 to July 1, 2005)
                      Jackie Quinteros (July 2 to August 17, 2005)
Technical Supervisor:  E. Ling Wong, BS, LATg
Supervisor, Necropsy: Angela Stahl, BS
Manager, Formulations: Gary Holley, BS
Manager, In-Life Operations: Michael R. Brunty, BS
Pathology Services: Meredith James, BS, LATG
Clinical Pathologist and Pathologist: James Szabo, DVM, PhD, DACVP
Vice President, Toxicology: Gary W. Wolfe, PhD, DABT
Director, Laboratory Animal Medicine: John Parrish, DVM, PhD, ACLAM

Ophthalmologist: Nancy M. Bromberg, VMD, MS, DACVO
                  Eye Clinic for Animals
                  6119 Massachusetts Avenue
                  Bethesda, Maryland 20816

Bioanalytical Chemistry: Jose Torres, MSc
                       (Formulation Stability,
                        Homogeneity, and Dose
                        Verification)
                       Industrial Organica, S.A. De C.V.
                       Ave. Almazan No. 100
                       Col Topo Chico
                       Monterey, Mexico

Sponsor: Howard Foundation Holdings Limited (Howard Foundation)
          Whitehill House
          Granhams Road
          Great Shelford
          Cambridge, CB2 5JY
          United Kingdom

Sponsor Representative/Study Monitor: David I. Thurnham, PhD
                                       Howard Professor of Human Nutrition
                                       Northern Ireland Center for Food and Health
                                       University of Ulster
                                       Coleraine BT52 1SA
                                       United Kingdom
STUDY PERSONNEL AND TEST SITES (CONTINUED)

Toxicology Operations:  
(Animal Facility)  
Gene Logic  
620 Professional Drive  
Gaithersburg, MD 20879

Laboratory Operations:  
(Clinical Pathology, Histology, Pathology)  
Gene Logic  
18761 North Frederick Avenue, Suite A  
Gaithersburg, MD 20879

Archives
Data:  
Iron Mountain, Inc.  
4451 Georgia Pacific Blvd., Suites L&M  
Frederick, MD 21704

Histology and Preserved Specimens:  
Charles River Laboratories, Preclinical Services - Pathology Associates (PAI)  
15 Worman's Mill Court, Suite I  
Frederick, MD 21701

Chemicals:  
Gene Logic  
18761 North Frederick Avenue, Suite A  
Gaithersburg, MD 20879
## STUDY TIMETABLE

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Initiation Date:</td>
<td>March 24, 2005</td>
</tr>
<tr>
<td>Experimental Start Date:</td>
<td>April 12, 2005</td>
</tr>
<tr>
<td>Receipt of Animals:</td>
<td>April 12, 2005</td>
</tr>
<tr>
<td>Randomization of Animals:</td>
<td>April 15, 2005</td>
</tr>
<tr>
<td>First Day of Dosing:</td>
<td>April 21, 2005</td>
</tr>
<tr>
<td>Last Day of Dosing:</td>
<td>July 19, 2005</td>
</tr>
<tr>
<td>Necropsy:</td>
<td></td>
</tr>
<tr>
<td>Terminal Sacrifice:</td>
<td>July 20, 2005</td>
</tr>
<tr>
<td>Recovery Sacrifice:</td>
<td>August 17, 2005</td>
</tr>
<tr>
<td>Experimental Completion Date:</td>
<td>July 10, 2006</td>
</tr>
<tr>
<td>Study Completion Date:</td>
<td>October 10, 2006</td>
</tr>
</tbody>
</table>
INTRODUCTION

The purpose of this study was to determine the toxicity of Mesozeaxanthin in male and female Han Wistar rats when administered once daily by oral gavage for 13 consecutive weeks, and to assess in a 4-week recovery period the delayed onset of any toxicity or persistence or reversibility of any effects noted earlier during the 13-week dosing phase. The rat was selected because it is the standard species for use in toxicology studies per FDA and ICH guidelines, and because this study was conducted in accordance with the regulatory guideline; alternatives could not be considered. The oral gavage approach was selected because it is the intended route of administration to humans.

Although mesozeaxanthin is probably not present in the human diet, it is found in the human eye and it is possible that its origin is from lutein in the diet. Lutein and mesozeaxanthin have slightly different structures but share a same chemical composition; both are found in the macular tissue of the human eye. The doses selected for the current study are based on similar doses as those used by Kruger et al (2002) who described a toxicity study on lutein at 2, 20 and 200 mg/kg/day. The top dose of Mesozeaxanthin in the current study was approximately 100,000 times higher (on a body weight basis) than the mean daily intake of natural zeaxanthin by humans (0.002 mg/kg/day).

The protocol, protocol amendments, and protocol deviations are presented in Appendix 15.

METHODS AND MATERIALS

Test and Control Articles

Neat Materials

The neat test and control articles used on this study are described in Text Table 1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Lot No.</th>
<th>Supplier</th>
<th>Purity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesozeaxanthina</td>
<td>5 11 M1 EPZ</td>
<td>Industrial Organica</td>
<td>Not provided</td>
<td>Orange, paste</td>
</tr>
<tr>
<td>Mesozeaxanthinb</td>
<td>5 09 J1 EPZ</td>
<td>Industrial Organica</td>
<td>Not provided</td>
<td>Orange, solid</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>UM0342</td>
<td>Spectrum Chemical</td>
<td>Not provided</td>
<td>Clear, yellow liquid</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>P8362344</td>
<td>ACH Food Company</td>
<td>Assumed 100%</td>
<td>Clear, yellow liquid</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>TV0947</td>
<td>Spectrum Chemical</td>
<td>Assumed 100%</td>
<td>Clear, yellow liquid</td>
</tr>
</tbody>
</table>

- 200 mg/mL; formulated in Corn Oil
- Used only for Week 13 formulations
The stock test article formulation, Mesozeaxanthin (200 mg/mL in Corn Oil), was supplied by Industrial Organica, Monterey, Mexico, and stored refrigerated (5 ± 3°C) and protected from light upon receipt. Three batches of the Corn Oil used at Gene Logic for animal dosing and used for preparation of test article dilutions were supplied by Spectrum Chemical Company, New Brunswick, NJ (2 batches) or ACH Food Company, Memphis, TN (1 batch). All batches were received and stored at room temperature upon arrival. Certificates of Analysis of the Mesozeaxanthin and Corn Oil, are presented in Appendix 1 and stability information of formulated Mesozeaxanthin are presented in Appendix 3.

Reserve samples of the test article (20 mL; ~ 36.756 grams), and control article (10 mL) were taken at Gene Logic prior to use on this study. The samples were archived at Gene Logic under the same conditions as the test and control articles.

Any remaining test and control articles were returned to the Sponsor following completion of the study.

Dose Formulations

The Mesozeaxanthin formulation (received from the supplier) was considered 100% pure for formulation purposes. Further dilution of the stock Mesozeaxanthin formulation for dosing purposes was adjusted based on a stated density of 0.9189 g/mL. Dose formulations were prepared weekly and used within 8 days after the stock formulation was diluted. Prior to use, the stock Mesozeaxanthin formulation was warmed overnight in a circulating water bath at 50°C (protected from light) and the Corn Oil was warmed in a circulating water bath at 50°C for approximately 20 minutes before used for dilution.

Dose formulations were prepared by adding the appropriate amount of the 200 mg/mL Mesozeaxanthin stock into a mortar, adding a small amount of Corn Oil and mixing into a paste, and then transferring the paste to a pre-calibrated beaker. A sufficient quantity of Corn Oil was added to achieve the desired final volume, and the formulation was then placed in a 50°C circulating water bath for 15 minutes and mixed (using a magnetic stir plate) for approximately 10 minutes until a suspension was attained. Following preparation, the total volume of each formulation was aliquoted into seven amber glass vials (one vial for use on each dosing day) and stored refrigerated (approximately 2 - 8°C) until used for dosing. Formulations were removed from the refrigerator and warmed in a water bath at approximately 40°C for at least 15 minutes and followed by mixing on a stir plate for at least 5 minutes during the dosing period.

Excess formulation was disposed of in accordance with Gene Logic SOPs, appropriate regulatory requirements, and information contained in the Material Safety Data Sheets.
Dosage Sampling

Triplicate 5-mL samples were taken from the top, middle and bottom portions of each dosing formulation in Week 1 for homogeneity analysis and dose verification. Five-mL samples of each dosing formulation prepared for Weeks 5, 9 and 13 were also collected for dose verification. The samples were stored refrigerated (5 ± 3°C) while protected from light and shipped to Industrial Organica, Mexico, on ice packs for homogeneity analysis (Week 1 formulations only) and/or dose verification.

Dosage Analysis

Analyses were conducted by Industrial Organica. A summary of the analytical method and the results are presented in Appendix 2.

Test Animals and Husbandry

Animals

Animal information is provided in Text Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species and Strain</td>
<td>Han Wistar Rats</td>
<td></td>
</tr>
<tr>
<td>Supplier</td>
<td>Charles River Laboratories</td>
<td></td>
</tr>
<tr>
<td>Number of Animals Received</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Number of Animals Used on Study</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Age of Animals at First Dose</td>
<td>7-8 weeks</td>
<td></td>
</tr>
<tr>
<td>Body Weight Range at First Dose</td>
<td>170.50-207.90 g</td>
<td>129.07-149.83 g</td>
</tr>
<tr>
<td>Disposition of Extra Animals</td>
<td>All ten extra animals (5/sex) were transferred into the training colony at the end of the in-life phase.</td>
<td></td>
</tr>
</tbody>
</table>

Animals were acclimated to laboratory conditions for 10 days prior to the first dose and released from quarantine by a staff veterinarian. During the acclimation period, each animal was identified by a temporary number that was recorded on each cage label.

Gene Logic’s Institutional Animal Care and Use Committee (IACUC) approved this protocol and found it to be in accordance with provisions of the USDA Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals, and the US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals.
Husbandry

Animal husbandry was provided as described in Text Table 3.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Certified Global Harlan Tekland Laboratory 2018 Rodent Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Water via an automatic watering system and water bottles</td>
</tr>
<tr>
<td>Bedding</td>
<td>Certified hardwood bedding</td>
</tr>
<tr>
<td>Caging</td>
<td>Polycarbonate cages</td>
</tr>
<tr>
<td>Racks</td>
<td>Stainless steel racks</td>
</tr>
<tr>
<td>Animals Per Cage</td>
<td>One</td>
</tr>
<tr>
<td>Temperature Range</td>
<td>64 to 79°F</td>
</tr>
<tr>
<td>Humidity Range</td>
<td>30 to 70%</td>
</tr>
<tr>
<td>Light Cycle</td>
<td>12-hour light/12-hour dark, interrupted as necessary for study-related events</td>
</tr>
<tr>
<td>Air Changes</td>
<td>Minimum of 10 air changes per hour</td>
</tr>
</tbody>
</table>

The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates and specific nutrients.

The water was routinely analyzed for contaminants and specific microbes.

The bedding was analyzed by the manufacturer for acceptable levels of heavy metals, aflatoxins, bacteria, yeasts, molds, and organophosphates prior to certification.

Feed and water were provided ad libitum, except on SD 90-91 (before terminal sacrifice) and SD 118-119 (before recovery sacrifice) when food-fasting was implemented and rats were fasted for 19-23 hours before termination at those 2 occasions. No contaminants were known to be present in the diet, water, or bedding at levels that might have interfered with achieving the objectives of the study.

Environmental controls were set to maintain animal room conditions as shown in Text Table 3. Actual temperature and relative humidity in the animal room or zone were monitored continuously by a computerized system and manually recorded at least once daily. All environmental parameters were maintained within the protocol requirements, except as noted in Appendix 15.

Experiment Design

Group Assignment and Doses

Animals were initially accepted into the randomization pool based upon body weights and physical examinations. Male and females were randomized separately. They were assigned to study groups using computer-generated random numbers. At randomization the mean body weight for each group was
not statistically different \((p<0.05)\) from the control mean. After the randomization process, each study animal was assigned a unique number and identified by a cage card and ear tag. Animals were assigned to groups as shown in Text Table 4.

Text Table 4: Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Test Article Dose Level (mg/kg/day)</th>
<th>Test Article Concentration (mg/mL)</th>
<th>Males</th>
<th>N</th>
<th>Animal Numbers</th>
<th>Females</th>
<th>N</th>
<th>Animal Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn Oil</td>
<td>0</td>
<td>0</td>
<td></td>
<td>15</td>
<td>20271-20285</td>
<td>15</td>
<td>20286-20300</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mesozeaxanthin</td>
<td>2</td>
<td>0.2</td>
<td></td>
<td>10</td>
<td>20301-20310</td>
<td>10</td>
<td>20311-20320</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mesozeaxanthin</td>
<td>20</td>
<td>2</td>
<td></td>
<td>10</td>
<td>20321-20330</td>
<td>10</td>
<td>20331-20340</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mesozeaxanthin</td>
<td>200</td>
<td>20</td>
<td></td>
<td>15</td>
<td>20341-20355</td>
<td>15</td>
<td>20356-20370</td>
<td></td>
</tr>
</tbody>
</table>

\(N = \) number of animals per group

Dose Administration

Dosing information is presented in Text Table 5.

Text Table 5: Dose Administration Information

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Oral gavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of Dosing</td>
<td>Daily</td>
</tr>
<tr>
<td>Duration of Dosing</td>
<td>SD 1 to 90</td>
</tr>
<tr>
<td>Dose Volume</td>
<td>10 mL/kg; based on most recent body weight</td>
</tr>
<tr>
<td>Equipment</td>
<td>3-mL or 5-mL syringes with 16-gauge/3-inch needles</td>
</tr>
<tr>
<td>Dosing Conditions</td>
<td>Animals were dosed at approximately the same time each day</td>
</tr>
</tbody>
</table>

Observations

Animals were observed as shown in Text Table 6.

Text Table 6: Animal Observations/Measurements

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Frequency of Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cageside Observations</td>
<td>≥ 2 Daily</td>
</tr>
<tr>
<td>Clinical Observations</td>
<td>SD1 (pre-dose), once weekly, and at terminal sacrifice</td>
</tr>
<tr>
<td>Body Weight</td>
<td>SD1 (pre-dose), once weekly, on SD 90 and 118 (non-fasted), and at scheduled sacrifice on SD 91 and 119 (fasted)</td>
</tr>
<tr>
<td>Ophthalmological Examinations</td>
<td>Prior to scheduled terminations (on all surviving animals)</td>
</tr>
</tbody>
</table>
Cageside observations included observation for mortality, moribundity, general health, and signs of toxicity. Clinical observations included evaluation of skin and fur characteristics, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavior patterns. Ophthalmological examinations were conducted using an indirect ophthalmoscope following 1% Tropicamide mydriasis.

**Clinical Pathology**

Blood was collected for clinical pathology evaluation as shown in Text Table 7. Animals were fasted overnight for 19-23 hrs (with water available) prior to sample collection.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chemistry</th>
<th>Hematology</th>
<th>Coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection Day</td>
<td>Prior to necropsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection Method</td>
<td>Through the retroorbital plexus, abdominal aorta or cardiac puncture when rats were under 70% CO$_2$/30% O$_2$ anesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume Collected</td>
<td>~ 1 mL</td>
<td>~ 0.5-mL</td>
<td>~ 1.8-mL</td>
</tr>
<tr>
<td>Tubes Used</td>
<td>2.5-mL serum separator</td>
<td>0.5-mL potassium EDTA tube</td>
<td>1.8-mL sodium citrate tube</td>
</tr>
</tbody>
</table>

Hematology and Coagulation samples were stored refrigerated and clinical chemistry samples were stored frozen before analysis. Blood samples were transported on ice packs to Gene Logic's Clinical Pathology Laboratory for analysis. Parameters evaluated and methods used are described in the Appendix 9.
Termination, Necropsy and Histopathology

Termination

On SD 91 (terminal sacrifice) and 119 (recovery sacrifice), all designated animals were euthanized by carbon dioxide inhalation followed by exsanguination.

Necropsy

Animals were necropsied as soon as possible after the time of death. A full gross necropsy, which included examination of the external surface of the body, all orifices, the cranial, thoracic, and abdominal cavities, and contents within each body cavity was performed. Protocol-specified organs were weighed as soon as possible after dissection; paired organs were weighed together. Bone marrow smears were prepared from the sternum; bone marrow slides were air dried, fixed in methanol, and stored for possible future evaluation. The eyes, together with optic nerves, hardierian and lacrimal glands, testes and epididymides, were fixed in modified Davidson’s fixative and transferred to 70% ethanol within 24-48 hours of collection. All other tissue samples and the animal identification (ear tag) were preserved in 10% neutral buffered formalin (NBF).

Histopathology

All tissue samples from the Groups 1 and 4 animals sacrificed following the treatment phase and the liver, kidneys, spleen, and stomach from Groups 2 and 3 animals were processed and evaluated. The liver, kidneys, spleen, and stomach from the recovery sacrifice animals were also processed and evaluated. Those tissue samples were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically by a board-certified veterinary pathologist.

Statistical Analyses

Body weights, body weight change, absolute and relative organ weights, and clinical pathology data were analyzed statistically.

Quantitative results were analyzed using the Kolmogorov-Smirnov test for normality, the Levene Median test for equal variance, and by one-way Analysis of Variance (ANOVA). If either the normality or equal variance test failed, then the analysis was continued using the non-parametric Kruskal-Wallis ANOVA on rank-transformed data. For parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunnett’s t-test was used to delineate which groups (if any) differed from the control. For non-parametric data, if the Kruskal-Wallis ANOVA indicated statistical significance among experimental groups then the Dunn’s test was used to delineate which groups (if any) differed
from the control. The probability value of less than 0.05 (two-tailed) was used as the critical level of significance for all tests.

Statistical analysis was conducted using SigmaStat™ Statistical Software, Version 1 (Jandel Scientific, San Rafael, California). Groups with sample sizes of 1 were excluded from statistical analysis. The term “significant” is used throughout the text of the report to indicate statistical significance at p<0.05.

Record Retention
All study data, including (but not limited to) animal data, clinical pathology data, necropsy data, histology and pathology data, professional reports, study protocol (including amendments), final study report, and any communications concerning the conduct of the study will be retained in the archive of Gene Logic for a period of 5 years following completion of the final report.

Preserved tissues, blocks, and slides will be maintained for the 5-year period at the archive facility at PAI.

Following the 5-year period (or before at Sponsor’s request), the Sponsor will be contacted to determine the disposition of these materials. All electronic data will be maintained at Gene Logic. Records regarding disposition of data and specimens will be maintained at Gene Logic.

Study data generated by the Sponsor or sub-contractors will be archived by the Sponsor or sub-contractors, respectively.
RESULTS

Stability of Test Article Formulations

Stability data of formulated test article are presented in Appendix 3.

Stability data provided by the Sponsor (generated at Industrial Organica) indicated that the 200 mg/mL stock formulation and the 0.2, 2.0, and 20.0 mg/mL formulations of Mesozeaxanthin were stable for up to 14 days after formulations when maintained at either 3-5°C or at 25°C.

Formulation Homogeneity and Dose Verification

Formulation homogeneity and dose verification data are presented in Appendix 4.

Analysis of dose formulations collected in Weeks 1, 5, 9, and 13 revealed that the test article was properly formulated and stable during formulation; mean test article concentration values ranged from 92.22 to 110.4% of target concentrations. No test article was detected in the control formulation. Homogeneity results measured on Week 1 samples indicated that the dose formulations were homogenous with relative standard deviations being <6% for all analyzed formulations.

Animal Disposition and Clinical Observations

Group and individual animal disposition and observation data are presented in Table 1 and Appendix 5, respectively.

No compound-related mortality or signs of toxicity were noted. Other observations noted included alopecia, abrasions, and hyperactivity; these observations were considered unrelated to treatment because they occurred in both the compound-treated and control groups or only appeared sporadically in low incidence throughout the study with no correlation to treatment or sex.

Body Weights and Body Weight Changes

Group summary and individual body weight and body weight change data are presented in Tables 2 and 3 and Appendices 6 and 7, respectively.

No compound-related body weight changes were noted. No significant differences were noted in absolute body weight or total body weight change over the course of the study for either sex. Statistically significant increases in weekly body weight changes were noted as follows: 20 mg/kg/day males on SD 22-29 and 43-50; 200 mg/kg/day males on SD 36-43; 2 mg/kg/day females on SD 43-50; and
200 mg/kg/day females on SD 22-29 and SD 71-78. These changes were considered incidental and unrelated to treatment because the changes were infrequent, sporadic, and not dose related.

**Ophthalmology**

The Ophthalmology Report is presented in Appendix 8.

No compound-related findings were noted ophthalmologically. A few findings were noted and listed in Text Table 8. Those changes noted were considered incidental and unrelated to treatment because the changes were noted in the control group as well as the high dose groups. These changes were infrequent, sporadic, not dose-related and/or without histopathological correlations.

<table>
<thead>
<tr>
<th>Terminal Sacrifice</th>
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<th>Observations</th>
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<td>20271</td>
<td>1M</td>
<td></td>
<td>Crystalline corneal opacities OD</td>
</tr>
<tr>
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<td>1M</td>
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<td>Crystalline corneal opacities OU</td>
</tr>
<tr>
<td>20283</td>
<td>1M</td>
<td></td>
<td>Crystalline corneal opacities OD</td>
</tr>
<tr>
<td>20286</td>
<td>1F</td>
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<td>Crystalline corneal opacities OU</td>
</tr>
<tr>
<td>20292</td>
<td>1F</td>
<td></td>
<td>Crystalline corneal opacities OD</td>
</tr>
<tr>
<td>20297</td>
<td>1F</td>
<td></td>
<td>Crystalline corneal opacities, anterior synechiation irregular pupil and retinal degeneration OS</td>
</tr>
<tr>
<td>20327</td>
<td>3M</td>
<td></td>
<td>Pinpoint crystalline corneal opacities OS</td>
</tr>
<tr>
<td>20353</td>
<td>4M</td>
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<td>Crystalline corneal deposits OD</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Recovery Sacrifice</th>
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<th>Group</th>
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</tr>
</thead>
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<td>Crystalline lens opacities OD</td>
</tr>
<tr>
<td>20297</td>
<td>1F</td>
<td></td>
<td>Crystalline lens opacities, Iritis, focal lens cataract and focal retinal OD; degeneration OS</td>
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<td>Crystalline lens opacities OU</td>
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<td>Lens opacity OS</td>
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</tr>
<tr>
<td>20367</td>
<td>4F</td>
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<td>Crystalline lens opacities OU</td>
</tr>
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**Clinical Pathology**

The Clinical Pathology Evaluation and Data Reports are presented in Appendix 9.

No compound-related changes in hematology, clinical chemistry, or coagulation were noted.
Review of the SD 91 data revealed significantly higher alkaline phosphatase (ALKP) activity for Groups 3 and 4 male rats when compared to male controls. Examination of the individual animal data revealed a moderate degree of variability in these values in all dose groups, including the controls, indicating that the differences between the controls and Groups 3 and 4 male rats were most likely the results of individual animal variability rather than a compound effect. Total bilirubin (TBIL) concentration for Group 2 female rats was significantly lower when compared to female controls; the difference was minimal in nature, inconsistent with a dose response, and neither biologically or toxicologically significant.

Review of the SD 119 (recovery) data revealed significantly higher sodium (NA), total protein (TPROT), and globulin (GLOB) concentrations for Group 4 male rats when compared to the male control rats. These differences were minimal in nature and had no biological or toxicological significance. Prothrombin time (PT) was significantly higher for Group 4 female rats when compared to female controls; the difference was minor and had no biological or toxicological significance.

**Gross Pathology**

Gross pathology data are presented in Table 4 and Appendix 10.

No compound-related macroscopic findings were noted.

Observations at **terminal sacrifice** included the following: sporadic and infrequent incidences of reddened/darkened mandibular lymph nodes in treated (2 and 200 mg/kg/day males and 20 mg/kg/day females) and control animals; brown and/or enlarged thymus in one treated male (2 mg/kg/day) and one treated female (200 mg/kg/day); a distended uterus in treated (2 and 20 mg/kg/day) and control females; a cystic ovary in one treated female (20 mg/kg/day); and a mottled liver in one control female.

Observations at **recovery sacrifice** included a reddened brain in one treated male (200 mg/kg/day Male); reddened mandibular lymph nodes in treated (200 mg/kg/day/sex) and control animals; and a reddened thymus in one control female.

All findings listed above were considered incidental because they occurred infrequently, in both treated and control animals, exhibited no dose relationship, and/or are associated with normal female reproductive cycling events.

**Organ Weights**

Organ weight data are presented in Tables 5, 6 and 7 and in Appendices 11, 12 and 13.
At terminal sacrifice, the following significant differences in absolute and relative organ weight data were noted: lower adrenal and/or adrenal/body weight ratios in all treated females; lower brain/body weight ratios in the 20 and 200 mg/kg/day females; and higher liver/brain weight ratio in the 20 mg/kg/day females.

At recovery sacrifice, the following significant differences in absolute and relative organ weight data were noted: lower thymus weight, heart/body weight ratio, thymus/body weight ratio, and thymus/brain weight ratio in the 200 mg/kg/day males. No significant differences were noted in the female data.

All organ weight changes noted above were considered incidental and unrelated to treatment, due to lack of dose responses and/or microscopic correlations.

**Histopathology**

The Histopathology Report is presented in Appendix 14.

No compound-related histopathology findings were noted.

Lesions considered to be spontaneous and incidental were observed in treated and control rats. These lesions consisted of early lesions of nephropathy (tubular regeneration; cortical, medullary and mucosal mononuclear cell infiltrates; and mineralization within the kidney); vacuolation within the adrenal gland; mononuclear cell infiltration within the hardener gland; hepatocellular vacuolation and mononuclear cell infiltration within the liver; acute hemorrhage within the lung, mandibular lymph node, and thymus; dilation of uterus; and mononuclear cell infiltration within the prostate. These lesions were noted sporadically, in low frequency, and/or were not dose-proportional, and are recognized as background findings of rats.

Some microscopic observations seen only in compound-treated animals were also considered to be spontaneous due to incidence and severity. On SD 91, focal, minimal, granulomatous inflammation within the liver in animal 20363 (4F); unilateral, pelvic dilation within the kidney in animal 20329 (3M); multifocal, minimal, histiocytosis within the lung in animal 20356 (4F); focal, minimal, perivascular mononuclear cell infiltrate within the pancreas and focal, minimal, luminal neutrophilic infiltrate within the prostate in animal 20348 (4M); multifocal, minimal, subacute inflammation within the stomach in animal 20301 (2M); multifocal, minimal, subacute hemorrhage within the thymus in animal 20357 (4F) and multifocal, minimal, mononuclear cell infiltrate with the lacrimal gland in animal 20344 (4M) were considered incidental and/or spontaneous. Multifocal, unilateral, subacute, mucosal inflammation within the kidney in animal 20348 (4M) and animal 20282 (1M), on SD 91 and SD 119, respectively, were also considered incidental and unrelated to the test article administration.
CONCLUSION

Under the conditions of this study, daily oral administration of Mesozeaxanthin at doses of up to 200 mg/kg/day was well tolerated in rats. The no-observed-adverse-effect-level (NOAEL) of Mesozeaxanthin in rats is >200 mg/kg/day when administered orally for 13 consecutive weeks.
REFERENCES

ABBREVIATIONS

Not all abbreviations listed are used in this report.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Abbreviation</th>
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<tr>
<td>↑</td>
<td>greater than control</td>
<td>S.D.</td>
<td>standard deviation</td>
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<tr>
<td>↓</td>
<td>less than control</td>
<td>RSD</td>
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<td>&gt;</td>
<td>greater than</td>
<td>TK</td>
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<td>&lt;</td>
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<td>PK</td>
<td>pharmacokinetic</td>
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<td>≥</td>
<td>greater than or equal to</td>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
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<td>maximum concentration</td>
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<tr>
<td>≈</td>
<td>approximately</td>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;</td>
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<td>degree</td>
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<td>%</td>
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<td>C</td>
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<td>QAU</td>
<td>Quality Assurance Unit</td>
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<td>rpm</td>
<td>revolutions per minute</td>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<td>LCA</td>
<td>Laboratory Corporation of America</td>
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<td>Pathology Associates, A Charles River Company</td>
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<td>RACB</td>
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25
CERTIFICATE OF ANALYSIS

Product: Hi Fil Z\(^*\) (Mesozeaxanthin concentrate)

Lot No.: 5 11 M1 EPZ

Date: March 11, 2005.

GUARANTEED ANALYSIS

Activity (gr/Kg.) by HPLC, 344.156

Carotenoid Composition by HPLC,

3R, 3'S Meso Zeaxanthin, grs/kg 210.13

3R, 3'R Zeaxanthin, grs/kg 52.53

Lutein, grs/kg 75.98

Free Xanthophylls, AOAC
Min. 95.0 % 99.12

Humidity:
Max. 5.0 % 1.7

Appearance: Golden orange paste

Sincerely,

M.Sc. Ricardo Montoya Olvera.

Industrial Orgánica, S.A. de C.V.
Ave. Almacén No. 100 Col. Topo Chico 94260 Apdo. Postal 1654 Monterrey, N.L., México
Tel. (81) 83-52-22-90 01-800 926-7000 Fax (81) 83-76-72-14 e-mail: icosa@att.net.mx
CERTIFICATE OF ANALYSIS

Product: Hi-Fil Z* (Mesozeaxanthin concentrate)
Lot No.: 5 09 J1 EPZ
Date: June 09, 2005.

GUARANTEED ANALYSIS

Activity (g/Kg.) by HPLC, 324.351

Carotenoid Composition by HPLC,

3R, 3'S Meso Zeaxanthin, g/Kg 207.02
3R, 3'R Zeaxanthin, g/Kg 51.75
Lutein, g/Kg 58.28
Xanthophylls Free, AOAC
Min. 95.00 % 99.00
Humidity:
Max. 5.0 % 0.6

Appearance: Golden orange paste

Sincerely,
M.Sc. Ricardo Montoya Olvera.
Quality Control

Industrial Orgánica, S.A. de C.V.
Ave. Almazán No. 100 Col. Topo Chico 64260 Apdo. Postal 1654 Monterrey, N.L., México
Tel. (81) 83-52-22-90 01-800 928-7000 Fax (81) 83-76-72-14 e-mail: ioca@att.net.mx