GENE LOGIC

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
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Gene Logic Study Number:
1567-04370

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

Study Completion Date:
October 10, 2006

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COMPLIANCE STATEMENT

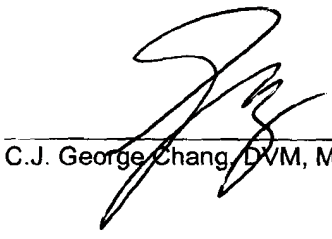
Thirteen-Week Oral (Gavage) Toxicity of Mesozeaxanthin
in Han Wistar Rats with a 4-Week Recovery

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

10/10/06

Date

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.

		Date Reported:	
Type of Audit	**Date Audited**	**Study Director**	**Management**
Protocol	April 13, 2005	April 13, 2005	April 13, 2005
SD 8 Dose Administration	April 28, 2005	May 3, 2005	May 3, 2005
SD 21 Dose Administration	May 11, 2005	May 18, 2005	May 18, 2005
Ophthalmology Exams	July 18, 2005	July 21, 2005	July 21, 2005
Necropsy	July 20, 2005	July 25, 2005	July 25, 2005
Raw Data/ Draft Final Report	December 1& 5-8, 2005	December 8, 2005	December 8, 2005
Histopathology Raw Data	December 12, 2005	December 13, 2005	December 13, 2005
Post/Final Report	July 5, 7 & 10, 2006	July 10, 2006	July 10, 2006

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

10/9/06
Date

SIGNATURE PAGE

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

Author:

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

10/10/06
Date

Peer Review:

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

10/9/06
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
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Supervisor, Necropsy:	Angela Stahl, BS
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Manager, In-Life Operations:	Michael R. Brunty, BS
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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

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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

Study Initiation Date:	March 24, 2005
Experimental Start Date:	April 12, 2005
Receipt of Animals:	April 12, 2005
Randomization of Animals:	April 15, 2005
First Day of Dosing:	April 21, 2005
Last Day of Dosing:	July 19, 2005
Necropsy:	
Terminal Sacrifice:	July 20, 2005
Recovery Sacrifice:	August 17, 2005
Experimental Completion Date:	July 10, 2006
Study Completion Date:	October 10, 2006

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.

Text Table 1: Neat Test and Control Articles

Name	Lot No.	Supplier	Purity	Description
Mesozeaxanthin[a]	5 11 M1 EPZ	Industrial Organica	Not provided	Orange, paste
Mesozeaxanthin[a]	5 09 J1 EPZ[b]	Industrial Organica	Not provided	Orange, solid
Corn Oil	UM0342	Spectrum Chemical	Not provided	Clear, yellow liquid
Corn Oil	P8362344	ACH Food Company	Assumed 100%	Clear, yellow liquid
Corn Oil	TV0947	Spectrum Chemical	Assumed 100%	Clear, yellow liquid

[a] - 200 mg/mL; formulated in Corn Oil [b] - Used only for Week 13 formulations

The stock test article formulation, Mesozeaxanthin (200 mg/mL in Corn Oil), was supplied by Industrial Organica, Monterry, 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 ($5 \pm 3°C$) 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.

Text Table 2: Animal Information

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

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.

Text Table 3: Husbandry

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

[a]The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates and specific nutrients.
[b]The water was routinely analyzed for contaminants and specific microbes.
[c]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 ($p \leq 0.05$) 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

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

N = number of animals per group

Dose Administration

Dosing information is presented in Text Table 5.

Text Table 5: Dose Administration Information

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

Observations

Animals were observed as shown in Text Table 6.

Text Table 6: Animal Observations/Measurements

Procedure	Frequency of Testing
Cageside Observations	≥ 2 Daily
Clinical Observations	SD1 (pre-dose), once weekly, and at terminal sacrifice
Body Weight	SD1 (pre-dose), once weekly, on SD 90 and 118 (non-fasted), and at scheduled sacrifice on SD 91 and 119 (fasted)
Ophthalmological Examinations	Prior to scheduled terminations (on all surviving animals)

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.

Text Table 7: Clinical Pathology

Parameter	Chemistry	Hematology	Coagulation
Collection Day	Prior to necropsy		
Collection Method	Through the retroorbital plexus, abdominal aorta or cardiac puncture when rats were under 70% CO_2/30% O_2 anesthesia		
Volume Collected	~ 1 mL	~ 0.5-mL	~ 1.8-mL
Tubes Used	2.5-mL serum separator	0.5-mL potassium EDTA tube	1.8-mL sodium citrate tube

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, harderian 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.

Anaylsis 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. Homogenity 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.

Text Table 8

Terminal Sacrifice

Animal ID	Group	Observations
20271	1M	Crystalline corneal opacities OD
20276	1M	Crystalline corneal opacities OU
20283	1M	Crystalline corneal opacities OD
20286	1F	Crystalline corneal opacities OU
20292	1F	Crystalline corneal opacities OD
20297	1F	Crystalline corneal opacities, anterior synechiation irregular pupil and retinal degeneration OS
20327	3M	Pinpoint crystalline corneal opacities OS
20353	4M	Crystalline corneal deposits OD

Recovery Sacrifice

Animal ID	Group	Observations
20296	1F	Crystalline lens opacities OD
20297	1F	Crystalline lens opacities, Iritis, focal lens cataract and focal retinal OD; degeneration OS
20298	1F	Crystalline lens opacities OU
20300	1F	Crystalline lens opacities OU
20351	4M	Crystalline lens opacities OD
20353	4M	Lens opacity OS
20354	4M	Crystalline lens opacities OD
20355	4M	Crystalline lens opacities OS
20367	4F	Crystalline lens opacities OU

OS - Left eye OD - Right eye OU - both eyes

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 harderian 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

Kruger, C.L., Murphy, M., DeFreitas, Z., Pfannkuch, F., Heimbach, J. (2002). An innovative approach to the determination of safety for a dietary ingredient derived from a new source: case study using a crystalline lutein product. Food & Chemical Toxicity 40:1535-49.

ABBREVIATIONS

Not all abbreviations listed are used in this report.

↑	greater than control	**S.D.**	standard deviation
↓	less than control	**RSD**	relative standard deviation
>	greater than	**TK**	toxicokinetic
<	less than	**PK**	pharmacokinetic
≥	greater than or equal to	**AUC**	area under the curve
≤	less than or equal to	**C_{max}**	maximum concentration
~	approximately	**$t_{1/2}$**	half-life
°	degree	**SD**	study day
%	percent	**GD**	gestation day
C	Celsius	**PND**	post-natal day
F	Fahrenheit	**i.p.**	intraperitoneal
L	liter	**i.v.**	intravenous
mL	milliliter	**s.c.**	subcutaneous
µL	microliter	**i.m.**	intramuscular
g	gram	**EPA**	Environmental Protection Agency
kg	kilogram	**FDA**	Food and Drug Administration
mg	milligram	**GLP**	Good Laboratory Practices
µg	microgram	**GMP**	Good Manufacturing Practices
ng	nanogram	**IACUC**	Institutional Animal Care and Use Committee
pg	picogram	**ICH**	International Conference on Harmonization
cm	centimeter	**MHLW**	Ministry of Health, Labor and Welfare
mm	millimeter	**NIEHS**	National Institute of Environmental Health Sciences
µm	micrometer	**NTP**	National Toxicology Program
sec	second	**OECD**	Organisation for Economic Co-operation and Development
min	minute	**PHS**	Public Health Service
h	hour	**QA**	Quality Assurance
d	day	**QAU**	Quality Assurance Unit
wk	week	**SOP**	Standard Operating Procedures
rpm	revolutions per minute	**USDA**	United States Department of Agriculture
NBF	neutral buffered formalin	**LCA**	Laboratory Corporation of America
No.	number	**PAI**	Pathology Associates, A Charles River Company
NA	not applicable	**RACB**	reproductive assessment by continuous breeding
N	number		

Industrial Orgánica

CERTIFICATE OF ANALYSIS

Product: Hi Fil Z® (Mesozeaxanthin concentrate)

Lot No.: 5 11 M1 EPZ

Date: March 11, 2005.

GUARANTEED ANALYSIS	LOT 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. Almazán No. 100 Col. Topo Chico 64260 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: iosa@att.net.mx

Industrial Orgánica

CERTIFICATE OF ANALYSIS

Product: Hi-Fil Z ® (Mesozeaxanthin concentrate)

Lot No: 5 09 J1 EPZ

Date: June. 09, 2005.

GUARANTEED ANALYSIS	LOT ANALYSIS
Activity (gr/Kg.) by HPLC,	324.351
Carotenoid Composition by HPLC,	
3R, 3' S Meso Zeaxanthin, grs/kg	207.02
3R, 3' R Zeaxanthin, grs/kg	51.75
Lutein, grs/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.
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