

**Statement of Safety for Zeaxanthin When Used as a
Dietary Supplement in a Water Soluble Beadlet
Formulation at a Level of Up to 10 mg/person/day**

Prepared for:

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a Water Soluble Beadlet Formulation at a Level of Up to 10 mg/person/day.**

Based on epidemiological evidence and biochemical considerations, it is believed that zeaxanthin has the capacity to minimize the risk of age related macular degeneration (AMD), the leading cause of irreversible loss of vision in the US. Dietary intake of zeaxanthin is known to lead to an increase of zeaxanthin in the macula of the eye in most individuals following prolonged exposure. The presence of zeaxanthin in the macula is critical given that, in theory, it can function to filter potentially damaging blue light, quench photo-chemically induced reactive oxygen species, attenuate chromatic aberrations and inhibit apoptosis. The question that epidemiological evidence cannot presently answer is what dietary intake level of zeaxanthin is optimal to adequately protect the eyes from AMD. As the answer is not currently ascertainable, a reasonable strategy for dietary supplementation is to use the highest zeaxanthin dose consistent with a high degree of safety.

A series of well-conducted safety studies are available, which provide an adequate basis for a safety assessment. Acute studies in rats and mice show a low order of acute toxicity with LD50 values greater than 4000 and 8000 mg/kg, respectively. Subchronic studies (90-day duration) were done in which the zeaxanthin beadlet formulation was administered in the diets of mice and rats at dose levels of 1000 mg/kg/day in rats and mice. Dogs were treated with a beadlet formulation in capsules at doses up to approximately 400 mg/kg daily. No clinical signs of toxicity, or adverse effect on clinical test parameters, gross abnormalities or organ weight changes at necropsy, or histopathological changes that could be associated with treatment were found in any of the studies. Thus, the no observed adverse effect level (NOAEL) in rats and mice was greater than 1000 mg/kg body weight/day and approximately 400 mg/kg body weight/day in dogs. A chronic study of 52 weeks duration, using a beadlet formulation, was performed in Cynomolgus monkeys with oral gavage doses of zeaxanthin of 0.2 and 20 mg/kg/day to groups of 2 male and 2 female monkeys. One additional male and female at 20 mg/kg/day were sacrificed after 6 months of treatment. Aside from coloration of the feces and adipose tissue, which are not considered an adverse effect, no clinical signs of toxicity, no adverse effect on clinical test parameters (hematology and clinical chemistry), gross abnormalities or organ weight changes at necropsy, or histopathological changes that are considered treatment related were found. Comprehensive evaluation of the eyes of treated monkeys, known to be an excellent model for humans, revealed no treatment related adverse changes after 52 weeks of treatment with the beadlet formulation. A dose related increase in zeaxanthin content in the retina was demonstrated, which is supportive of the intended use of zeaxanthin as a dietary supplement. In reproductive toxicity studies, there was no evidence of maternal toxicity, fetal toxicity or teratogenicity in treated rats or rabbits at doses up to 1000 mg/kg/day and 400 mg/kg/day, respectively.

In keeping with the strategy stated above to use the highest level of zeaxanthin consistent with safety, calculation of an upper safe level of zeaxanthin based on a conventional safety evaluation using a traditional 100-fold safety factor is set forth as follows:

Safety factors are calculated as the ratio between the NOAEL derived from the safety studies and the anticipated exposure of the material.

The lowest NOAEL for zeaxanthin is 20 mg/kg from the 52-week monkey study, which would represent an intake of 1200 mg/day for a 60 kg individual (60 kg X 20 mg/day = 1200 mg/day).

The average dietary intake of zeaxanthin in the US ranges from 0.1 to 0.4 mg/day.

The highest quintile of dietary intake of zeaxanthin in the US ranges from 0.9 to 1.9 mg/day.

Safety Factors (calculated based on a 60 kg individual)

**Supplement intake
(10 mg/day)**

1200 mg/day ÷ 10 mg/day = 120 X Safety factor

**Supplement intake + Average dietary intake
(10 mg/day + 0.4 mg/day = 10.4 mg/day).**

1200 mg/day ÷ 10.4 mg/day = 115 X Safety factor

**Supplement intake + highest quintile of average dietary intake
(10 mg/day + 1.9 mg/day = 11.9 mg/day)**

1200 mg/day ÷ 11.9 mg/day = 101 X Safety factor

As the margin of safety for the use of zeaxanthin as a dietary supplement at a dose of 10 mg/day for a 60 kg individual is at least 100 fold including the average dietary intake of the highest quintile, it is concluded that daily consumption of zeaxanthin at a level of 10 mg represents a safe use of a dietary supplement. Further, we believe the available safety data are sufficient to conclude that zeaxanthin is safe at a daily dose of 10 mg within the meaning of FDA's definition of safety for food ingredients as set forth in 21 CFR 170.3(i) "safe or safety means that there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use."

There are two supporting documents entitled:

- (1) Summary of Safety Studies Conducted with Zeaxanthin (Ro 01-9509) Water Soluble Beadlet Formulation.
- (2) Dietary Intake and Retinal Concentration of Carotenoids and Calculation of Safety Factors for Zeaxanthin (Ro 01-9509) Water Soluble Beadlet Formulation.

The information in these documents supports the conclusion that zeaxanthin does not present a significant or unreasonable risk of illness or injury under the intended conditions of use.

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R. Michael McClain, PhD. (Abbreviated CV)*

Present Positions (1998 to present)

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Previous Experience (1970 to 1998)

Toxicologist, Department of Toxicology and Pathology, Hoffmann La Roche Inc, Nutley NJ.
Experience in teratology and reproductive toxicology, general toxicology and carcinogenicity testing. **Positions:** Director of Toxicology, Research Advisor, Distinguished Research Leader.

Education

BS, Pharmacy (1965), MS, Pharmacology and Toxicology (1967), Duquesne University, Pittsburgh, PA.
PhD, Pharmacology (1970), University of Iowa, Iowa City, IA.

Professional Certification

Diplomate, American Board of Toxicology.
Fellow, Academy of Toxicological Sciences.

Professional Societies

Society of Toxicology (President, 1998).
American Association for the Advance of Science, NY Academy of Sciences.

Research Interests

Reproductive toxicology, mechanisms of toxicity, mechanisms of carcinogenicity (especially, adrenal, thyroid and hepatic), cancer risk assessment.

Publications and Presentations

52 full publications, book chapters, or editor.
104 invited presentations.

Scientific Advisory Boards

Dr. McClain has served on a number of Scientific Advisory Boards for the National Institutes of Environmental Health Sciences (NIEHS) the US Environmental Protection Agency (EPA) and has been a member of several Working Groups for the International Agency for Research on Cancer (IARC).

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W. Gary Flamm received his Ph.D. in Biochemistry from the Medical School of the University of Cincinnati in 1962. He also holds degrees in Pharmacy and Pharmaceutical Chemistry. Dr. Flamm is a Fellow of the American College of Toxicology and the Academy of Toxicological Sciences. After retiring from FDA in 1988, Dr. Flamm has spent the past 14 years as an independent consult specializing in safety evaluation in general and FDA matters in particular. Dr. Flamm's career in the U.S. Public Health Service spanned 25 years at the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) where he became extensively involved in health and safety evaluation of chemicals and their regulations. Dr. Flamm held several high-level positions at FDA including Chief Science Advisor to the Commissioner of FDA and Director of the Office of Toxicological Sciences of the Center for Food Safety and Applied Nutrition (CFSAN). Dr. Flamm is a recognized international authority in risk/safety evaluation of carcinogens and other toxic agents and has been called upon many times to testify before the U.S. Congress on the safety of substances in the food supply. He is a former President of both the American College of Toxicology (ACT), and the International Society for Regulatory Toxicology and Pharmacology (ISRTP) and has served on the council and held offices in the Society for Risk Analysis (SRA) and the Environmental Mutagen Society (EMS) and was program chairman for the Toxicology Forum for a number of years. He has served on the editorial boards of about a dozen journals addressing toxicologic and risk assessment subjects and has published over 120 papers in scientific journals and books and has held academic appointments in several major universities including California Institute of Technology, University of Edinburgh, Scotland and University of North Carolina. Dr. Flamm's career accomplishments in establishing a better understanding between government and industry in areas of safety evaluation have been recognized by both professional societies and government *via* such awards as the George H. Scott Award from the Toxicology Forum, the Superior Service Award from the Public Health Service, and the Recognition Award from the Environmental Mutagen Society.