Evaluation of the Effects of Neptune Krill Oil ™ on UVB-Radiation Induced Skin Cancer

Final Report to:

NEPTUNE
TECHNOLOGIES & BIORESSOURCES INC.

Submitted by:
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Introduction

Euphasia Pacifica, commonly known as Krill, make up the order of Euphausiacea, subclass of Malacostraca, class of Crustacea and phylum of Anthropoda. They are small shrimp or prawn-like crustaceans, habitants of oceans off the West coast of Vancouver Island, Russia, Ukraine and Japan. Approximate area of distribution at the Circum-polar in Antarctic waters is 35 million square kilometers. Approximately 90 species have been recognized, most of which range in length from 8 to 70 mm (mean length 16 mm.). Considering a population size of 500 million tones, Krill are the most important Zooplankton group in the world oceans after Copepods. Major predators are Baleen whales (blue, fin, mink), crabeater seals, fur seals, Adelie and macaroni penguins, petrels, fulmars and shearwaters, squid and fish. In Japan humans consume them as an ethnic delicacy.

NKO™ is extracted from the body of Krill with a innovative process of lipid extraction which produces a dehydrated residue. The most significant components of NKO™ are the Omega-3 fatty acids, containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), all-trans retinol or vitamin A, vitamin E, Astaxanthin and Canthaxanthin. Scientific evidence suggests that Omega-3 and antioxidants may have both preventive and

therapeutic properties for systemic disease, mainly cardiovascular and neoplastic.

Neoplastic disease is the second most frequent cause of morbidity and mortality in North America. In the year 2000, mortality is expected to rise to 552,200 Americans, which amounts to over 1,500 people per day. The incidence of malignancies is expected to rise to 1,220,100 this year. Since 1990, near 13 million new cases have been diagnosed excluding non-invasive cancers and squamous cell skin cancers. The incidence of squamous cell skin cancer alone within the year 2000 is expected to rise to 1.3 million (Source: American Cancer Society).

Since 1993 it has been observed that cancer mortality is decreasing while the incidence is increasing. Therefore, the number of cancer survivors is rising along with a respective rise in morbidity. Consequently, the health resources consumed by neoplastic disease are progressively elevating. According to the Pharmaceutical Industry Profile for the year 2000, reported by the Pharmaceutical Research and Manufacturers of America, 14,869 million dollars are spent on prescription drugs for neoplastic disease alone, covering 19.7% of the annual market.

Ultraviolet radiation plays an unequivocal role in the etiology of skin cancers such as basal and squamous cell carcinoma and melanoma. Exposure of skin to ultraviolet radiation can cause both short and long term adverse effects. The short-term effects are sunburn and erythema due to photosensitivity of skin. The long-term effects include aging and carcinogenesis. Studies suggest

that UVR-induced inflammation is caused by an increased production of prostaglandins and cytokines. UVR generates free radicals, which cause membrane damage, including skin damage. Retinols have been proven to have prophylactic effects against UV radiation induced skin cancer (Tsambaos D., Sampalis F: UV-radiation induced skin cancer: Inhibition by oral arotinoids. Gior. Ital. Chir. Dermatol. Oncol. 2:409 - 412, 1987).

Oxidation is the chemical process by which an ion from an atom or molecule steals of one or more of another's electrons altering the chemical structure irreversibly. The chemicals that exhibit this tendency for stealing electrons are referred to as oxidizing agents. We are constantly exposed to oxidative stress in our everyday environment by air pollution, tobacco smoke, exposure to chemicals, and exposure to ultraviolet (UV) light or other forms of ionizing radiation (Møller *et al*. 1996; Papas 1999). These oxidants change the chemical structure of DNA and proteins causing various pathological conditions including aging (Harman 1981; Ames and Shigenaga 1992), atherogenesis (Steinberg *et al*. 1989; Esterbauer *et al*. 1992), and carcinogenesis (Moody and Hassan 1982; Marnett 1987; Breimer 1990). Antioxidants protect the human body from oxidative damage by scavenging of radicals to prevent or terminate chain reactions and quenching of singlet oxygen and dissipating the energy as heat. astaxanthin has been proven to be twice as effective as beta-carotene (and about 80 times more effective than vitamin E) in quenching singlet oxygen in chemical solution (Di Mascio *et al* . 1991); and about 50% more effective than beta-carotene and zeaxanthin, in preventing fatty acid peroxidation in chemical solution (Terao 1989). In a membrane model, astaxanthin was found to be more

effective at scavenging peroxyl radicals than was beta-carotene (Palozza and Krinsky 1992).

A systemic photoprotective agent, which would neutralize free radicals, could prevent UVR-induced skin damage and short as well as long term adverse events. Neptune Krill Oil™ (NKO™) is a marine oil composed of a natural mixture of essential nutrients. It is characterized by its high content of phospholipids with substantially high quantities of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) both occupying the two fatty acid chains of the same molecule and potent antioxidants comprised of vitamins A and E, esterified astaxanthin, and a marine source novel flavonoid.

Omega-3 polyunsaturated fatty acids (EPA & DHA) and Omega-9 (Oleic acid) along with the high content of antioxidants in NKO™ justify further investigations of possible anticarcinogenic properties of a NKO™ preparation. The objective of this trial was to evaluate the photoprotective potential of NKO™ against UVB-induced skin cancer.

Materials and Methods

This was a randomized controlled pre-clinical trial. The animal model used was C57BL6 Nude Congenic Mice – B6NU-T heterozygotes because of the species-specific genetic susceptibility to skin cancer.

Mice were kept in a controlled environment with a 12 hour regulated light – dark cycle. In order to obtain an acceptable significant reduction = 25%

(δ = 0.33), statistical significance of 95% (P ≤ 0.05) and a certainty of 80%, 96 mice were included in the study.

Male and female mice were divided equally. In order to establish efficacy of NKO™ for the prevention of skin cancer, the test was conducted as a randomized double blind controlled trial. The mice were randomized in two groups of 48 mice. One group was treated with NKO™ and the other with active control or Soya oil. Each group was subdivided in three subgroups of 16 mice each depending on the mode of treatment; oral, topical or topical/oral respectively. The diet of the orally treated groups was supplemented with 10% of their daily intake with NKO™ or Soya oil. The daily oral dose given to the mice was equivalent to that of 2 g of NKO™ per day for a 70 kg man or a 60 kg woman. All mice were exposed to 30 minutes of UVB radiation per day. The distance between the mice and the lamps was set at 30cm. The dosage of radiation exposure was calibrated daily. According to animal specifications, the maximum time of exposure required for the development of cutaneous malignancy is 20 weeks at which time all remaining animals were euthanized by ether. All specimens were histologically examined for the presence of malignant pathology.

Results

Table 1: Outcome by Group and Method of Administration

Group	Outcome						Total	
	Cancer		Pre-Malignant		Normal			
	N	%	N	%	N	%	N	%
Krill Oral	**3**	**18.8%**	**3**	**18.8%**	**10**	**62.5%**	**16**	**100.0%**
Krill Topical and Oral	3	18.8%	5	31.3%	8	50.0%	16	100.0%
Krill Topical	2	12.5%	5	31.3%	9	56.3%	16	100.0%
Placebo Oral	6	37.5%	3	18.8%	7	43.8%	16	100.0%
Placebo Oral and Topical	6	37.5%	2	12.5%	8	50.0%	16	100.0%
Placebo Topical	6	37.5%	5	31.3%	5	31.3%	16	100.0%
Total:	26	27.1%	23	24.0%	47	49.0%	96	100.0%

Statistical Significance Testing for Incidence of Cancer

1. Krill Oral vs. Krill Topical vs. Krill Oral/Topical: ***P = 0.42***

Clinical Significance Testing for Incidence of Cancer

1. Krill Oral vs. Placebo Oral: ***49.7% reduction of incidence***
2. Krill Topical vs. Placebo Topical: ***49.7% reduction of incidence***
3. Krill Oral/Topical vs. Placebo Oral/Topical: ***66.6% reduction of incidence***

Table 2: Outcome by Overall Treatment Group

Group	Outcome						Total	
	Cancer		Pre-Malignant		Normal			
	N	%	N	%	N	%	N	%
Krill	8	16.7%	13	27.1%	27	56.3%	48	100.0%
Placebo	18	37.5%	10	20.8%	20	41.7%	48	100.0%
Total:	26	27.1%	23	24.0%	47	49.0%	96	100.0%

Overall Statistical Significance Testing for Incidence of Cancer

Krill vs. Placebo : ***P = 0.04***

These results show that overall Neptune Krill Oil™ significantly prevents the incidence of skin cancer. The analysis comparing different modes of administration showed that all three methods were effective within clinical significance. A different study would need to be designed for the specific evaluation of the three different modes of administration, in order to determine the preferred application method of choice.

Conclusion

The results of the present study clearly indicate that Neptune Krill Oil™ can significantly prevent skin cancer induced by chronic exposure to ultraviolet radiation.