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Safety assessment of Superba™ krill powder: Subchronic toxicity study in rats

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ABSTRACT

The safety of krill powder was assessed in a subchronic 13-week toxicity study where rats were fed krill powder or control diets. The krill powder inclusion in the test diet was 9.67% (w/w). There were no differences noted in body weight or food consumption in either gender. Differences in clinical chemistry values were noted in the krill powder-treated animals, but these findings were of no toxicological significance. A significant decrease in absolute heart weight, but not relative heart weight, was observed in both sexes given krill powder, although no corresponding histological changes were observed. Hepatocyte vacuolation was noted histologically in males fed krill powder. This finding was not associated with other indications of hepatic dysfunction. The no observed adverse effect level (NOAEL) for the conditions of this study was considered to be 9.67% krill powder.

1. Introduction

The two omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been proven to have a wide range of beneficial effects, in particular on cardiovascular health [1–5]. Fish and seafood intake is considered too low in a large proportion of the population in the Western world, and to take omega-3 food supplements is a way to improve one's daily need of these important fatty acids. To date, fish oils have been the most traditional omega-3 supplements, but new sources of omega-3 fatty acids, like algae and krill, are gaining popularity [6–8].

Krill are shrimp-like crustaceans that are harvested commercially in the Antarctic Sea [9]. The estimated amount of krill (*Euphausia superba*) in Antarctica is between 125 and 750 million metric tonnes (http://www.fao.org/fishery/species/3393/en), being one of the most abundant animals on the planet. There are currently two main products produced from krill: krill oil and krill powder. Krill oil is sold as a food supplement and is characterised by a large proportion of phospholipids, especially phosphatidylcholine (PC) [10]. The majority of EPA and DHA in krill oil is esterified into PCs and omega-3 fatty acids in phospholipid form have been shown to be efficiently taken up by body tissues [11–14]. Also krill powder consists of a large fraction of phospholipids (20.2%) and it further contains proteins (41.7%) in addition to a lipid fraction (51.7%).

Besides the high presence of phospholipids, krill also contains the red pigment molecule astaxanthin [15]. Astaxanthin is an antioxidant carotenoid that gives krill powder its reddish colour.

The product has been used for both human and animal dietary supplementation [16–18]. So far, krill powder has been tested in two pre-clinical [17,18] and one clinical study [16]. The pre-clinical studies investigated the

Abbreviations: ANOVA, analysis of variance; ANCOVA, analysis of covariance; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; KP, krill powder; NOAEL, no observed adverse effect level; SO, soya bean oil.

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effect of krill powder on hepatic gene regulation in healthy mice [17] and on inflammation and lipid metabolism in mice overexpressing TNFα [18]. The clinical study examined krill powder supplementation in mildly obese men and its effect on fat distribution, blood lipid levels and the endocannabinoid system [16].

The objective of the present study was to assess the safety of krill powder in a 13-week subchronic toxicity study in Wistar rats.

2. Materials and methods

2.1. Test materials

Superba™ krill powder was provided by Aker BioMarine Antarctic AS (Oslo, Norway). The raw material was analysed for fatty acid composition, total lipid, lipid classes, proteins, ash, salt and astaxanthin content (Nofima AS, Bergen, Norway). The composition of the krill powder is shown in Table 1. The amino acids profile of krill powder has been analysed previously [17].

Table 1
Composition of krill powder.

Compound	% in krill powder
Total lipids	51.7
Phospholipids	24.7
Phosphatidylcholine	22.7
Phosphatidylethanolamine	1.7
Cholesterol esters	0.5
Triacylglycerol	25.3
Diacylglycerol	0.8
Proteins	41.7
Ash	5.4
Salt (NaCl)	2.1
Astaxanthin (mg/kg)	115.5
Fatty acids	
14:0	6.0
16:0	8.9
18:0	0.6
20:0	0.1
22:0	n.d.
16:1 n-7	3.1
18:1 n-9 + n-7 + n-5	7.9
20:1 n-9 + n-7	0.5
22:1 n-11 + n-9 + n-7	0.2
24:1 n-9	0.1
16:2 n-4	0.3
16:3 n-4	0.2
18:2 n-6	1.0
18:3 n-6	0.2
20:2 n-6	0.1
20:3 n-6	0.1
20:4 n-6	0.2
22:4 n-6	n.d.
18:3 n-3	0.5
18:4 n-3	1.3
20:3 n-3	n.d.
20:4 n-3	0.2
20:5 n-3	6.2
21:5 n-3	0.2
22:5 n-3	0.2
22:6 n-3	2.7
SFA	15.7
MUFA	11.6
Omega-6 PUFA	1.4
Omega-3 PUFA	11.2

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n.d.: not detected. Lower detection limit for fatty acid measurements was 0.1 g/100 g. All amounts shown are gram per 100 g of krill powder, except for astaxanthin which is in mg/kg.

2.2. Subchronic toxicity study

The subchronic toxicity study was designed and conducted based on the regulatory guidelines OPPTS 870.3100, OECD No. 408 and US FDA Redbook. Twenty male and twenty female Han Wistar rats were obtained from Charles River UK Limited. They were acclimatised for a period of 14 days. The study was performed at Charles River, Tranent, Edinburgh, UK. The animals were approximately seven weeks old at treatment start and were in the weight range of 179–229 g (males) and 109–162 g (females). Animals were randomised to cages on racks separated by treatment group and sex and housed in the same room. Control and krill powder groups were housed on separate racks with two to three animals per cage. Rats were given food and water (domestic mains water) *ad libitum* during this period, and were provided with wooden chew sticks for environmental enrichment (Tapvei Estonia OÜ, Harjumma, Estonia). The animals were kept at 19–23 °C, 40–70% humidity and a fixed light cycle (light hours were from 7 to 19 h) throughout the study period. The study was conducted in accordance with the OECD Principles of Good Laboratory Practice (GLP) as incorporated into the United Kingdom Statutory Instrument for GLP, and as accepted by regulatory authorities throughout the European Community, United States (FDA and EPA) and Japan (MHLW, MAFF and METI).

2.3. Treatment

Two groups of ten male and ten female Han Wistar rats were fed diets containing a total of 8% oil for a period of 13 weeks. The control diet was supplemented with 8% soya bean oil. The krill powder diet was incorporated with 9.67% krill powder (corresponding to 5% krill oil). The krill powder contained 20.2% PL, 51.7% total lipids, 41.7% proteins and 115.5 mg/kg astaxanthin (for more details see Table 1), and the amounts of soy bean oil (3%) and casein added to the krill powder diet were reduced in such a way that the lipid content and protein content were the same in the two test diets. This amount of krill powder is equal to inclusion of 5% krill oil, which corresponds to 2.5–5 g/kg of body weight. After conversion to human equivalent doses (HED), the studied dose range provides a 24- to 48-fold safety margin with the recommended supplement level of 1 g/day.

The diets were based on the standard RM1 diet (http://www.sdsdiets.com/pdfs/RM1P-E-FG.pdf) and prepared by Special Diet Services (Witham, UK) according to their in-house standard operating procedures.

The krill powder diet was verified for homogeneity by Nofima AS (Bergen, Norway). After inclusion of krill powder into the final diet form, the recovery of EPA and DHA was 97.0 ± 0.7% and 96.8 ± 0.7%, respectively. The