



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.

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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 TNF α [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].

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, Harjumaa, 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),

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.

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

measurements were performed at the beginning of the study and the homogeneity was verified by measuring EPA and DHA in 3 samples of the krill powder diet. Both diets were stored at -20°C to ensure stability of the feed until given to the animals on a daily discard and top-up routine.

2.4. Observations, body weight and food consumption

Every day during the study the well-being and reaction to treatment of the animals was monitored and once each week the animals received a detailed clinical examination. The eyes of the animals were examined before, during and at the end of the experiment. Body weights were recorded weekly and the food consumption by each cage of animals was measured and recorded daily throughout the treatment period. Water consumption was qualitatively evaluated by visual inspection every week.

2.5. Haematology, clinical chemistry and urinalysis

At the termination of the study, blood samples for haematology and clinical chemistry were obtained from all surviving animals. Samples were obtained from non-fasted animals *via* the orbital sinus under isoflurane anaesthesia. 0.5 mL whole blood was transferred into EDTA tubes for measurement of haematology parameters using the ADVIA 120 automated haematology analyser (Bayer, Munich, Germany). Haemoglobin, red blood cell count, haematocrit, white blood cell count, mean cell volume, mean cell haemoglobin concentration, platelet count, reticulocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unclassified cells were quantified. Prothrombin time and activated partial thromboplastin time were measured in trisodium citrate-treated blood (blood:citrate ratio of 9:1), with an ACL Advance coagulation analyser (Diamond Diagnostics, MA, USA). Lithium heparin tubes were used for blood collected for clinical chemistry. The tubes were centrifuged and analysed with a Roche P module clinical chemistry analyser using a Roche test kit (Roche, Basel, Switzerland) for urea, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, cholesterol, total bilirubin, calcium and phosphate. Sodium and potassium was analysed using a Roche P module clinical chemistry analyser with an indirect ion selective electrode. Globulin was calculated by subtraction of the albumin concentration from the total protein concentration; albumin:globulin ratio was calculated by $(\text{albumin})/(\text{total protein} - \text{albumin})$. During week 13, urine samples were collected over a 4-h period from all animals. They were deprived of food and water and housed individually in metabolic cages. The following measurements were performed in fresh urine: volume (weighing of urine sample), specific gravity (manual assessment using a refractometer), colour, pH, protein, glucose, ketones, urobilinogen, bilirubin, pigments (Aution JET 9UB test strips using an Aution Jet AJ4270 analyser, Menarini Diagnostics, Florence, Italy) and microscopy of the spun deposits (epithelial cells, crystals, white blood cells, red blood cells, organisms, casts, other abnormalities).

2.6. Neurobehavioral studies

During week 12 or 13, detailed neurotoxicological observations were performed on all animals, including parameters of a functional observation battery. Most of the assessments were based on scaled observations of the animals' behaviour/status and included home cage and open field evaluations. Moreover, condition of the eyes and coat, presence of salivation, ease of removal from cage, body temperature, and overall ease of handling were recorded. For a period of two minutes, open field observations in a standardised arena concentrated on: rearing, grooming, latency, level of mobility, urination/defecation, tremor/convulsions, vocalisation, arousal, posture, pilo-erection, palpebral closure, gait abnormalities, stereotypy and/or unusual behaviours. At a standardised time of the day, reactions to sound and touching the rump with a blunt probe were also observed. Landing foot splay, motor activity, grip strength (using a method derived from Meyer et al. [19]) and pain perception (using a method derived from D'Amour [20]) were included as quantitative measurements.

2.7. Pathology

Non-fasted animals were sacrificed in a random order by exsanguination after anaesthesia with carbon dioxide after 13 weeks of treatment. Body weight of each animal was recorded, followed by severance of major blood vessels. All animals were subjected to a detailed necropsy examination and more than 40 different tissues were subject to a more comprehensive histopathological examination. A complete external and internal examination, which included body orifices, respiratory tract and cranial, thoracic and abdominal cavities, was performed. Representative tissues were fixed in 10% neutral buffered formalin or Davidson's fluid (only eyes, optic nerve and testis): abnormal tissue, adrenal glands, aortic arch, blood smear, brain, eyes, epididymis, gastro-intestinal tract, harderian gland, heart, implant, kidney and ureter, liver, lung, mesenteric lymph node, nasal cavity, oesophagus, optic nerve, ovaries, pancreas, pituitary, prostate, rib, salivary glands, sciatic nerve, seminal vesicles, spinal cord, skin and mammary gland, spleen, sternum, submandibular lymph node, testis, thigh muscle, thyroid with parathyroid, tongue, trachea, urinary bladder, thymus, uterus and vagina. Sections were cut 4–6 μm thick, and stained with haematoxylin and eosin (H&E) (unless otherwise stated) and evaluated by a pathologist. The following organs were weighed: adrenal glands, brain, epididymides, heart, kidneys, liver, lung, ovaries, pituitary gland, prostate, spleen, testes, thymus and thyroid.

2.8. Statistical analysis

Unless otherwise stated, all statistical tests were two-sided and performed at the 5% significance level using in-house software and performed as described below. Pairwise comparisons were performed between the krill powder and the control group for males and females separately. Quantitative data, body weight, food consumption,

haematology, coagulation, clinical chemistry, urinalysis, motor activity and quantitative functional observational battery measurements were analysed for homogeneity of variance using the 'F-Max' test. If the group variances appeared homogeneous, a parametric ANOVA was used and pairwise comparisons made using Fisher's F protected LSD method *via* Student's *t*-test, *i.e.* pairwise comparisons were made only if the overall *F*-test was significant. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a Kruskal–Wallis non-parametric ANOVA was used and pairwise comparisons made using chi squared protection (*via* *z* tests, the non-parametric equivalent of Student's *t*-test). Organ weights were analysed using ANOVA as above and by analysis of covariance (ANCOVA) using terminal body weight as covariate. In addition, organ weights as a percentage of terminal body weight were analysed using ANOVA as above. Histological incidence data were analysed using Fisher's Exact Probability Test.

3. Results

3.1. Mortality

There were two animals sacrificed prematurely during the study. One male animal in the control group was euthanized on Day 81 of the study having previously

displayed clinical observations including abnormal respiration, weight loss, and a subcutaneous mass on left ventral abdomen. A mammary adenoma was observed by histological examination, which could explain the subcutaneous mass observed at necropsy. Another male animal in the krill powder group was euthanized on Day 38 due to an open and wet lesion on dorsal neck. Histologically, focal ulcerative dermatitis was observed, which correlated to the raw data observed at necropsy.

3.2. Observations, body weight and food consumption

During the 13-week study period, there were no notable clinical signs that could be related to krill powder treatment. All animals given a krill powder diet, however, were noted to have abnormal pale and/or yellow coloured faeces. This was considered to be a result of the presence of astaxanthin in the krill powder (11.2 mg/kg diet) and not to be of toxicological significance [21]. Body weights in both sexes throughout treatment, were not statistically different between the control and krill powder groups (Fig. 1). The food consumption (g/animal/day) in control and krill powder group was measured weekly for both sexes (Fig. 2), and were not statistically different between the control and krill powder groups. Throughout treatment, the overall mean intake of krill powder was 5357 mg krill powder/kg body weight/day for males and 6284 mg krill powder/kg body weight/day for females (dosages calculated from data in

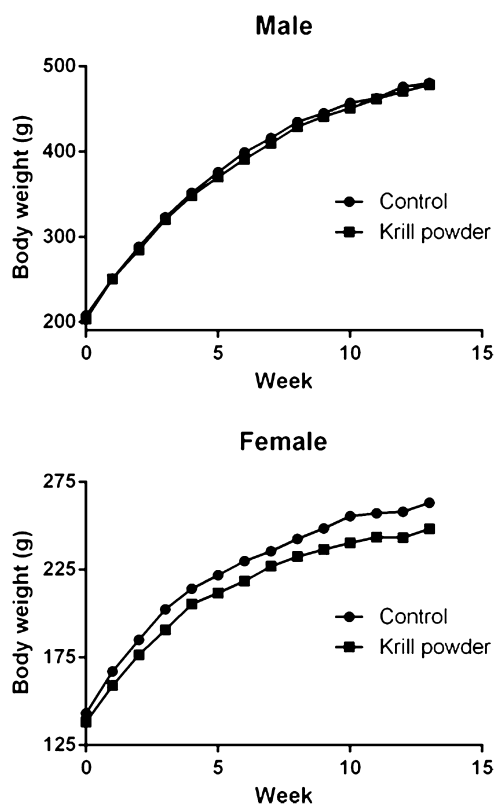


Fig. 1. Group mean body weights of male and female Han Wistar rats fed diets containing krill powder for 13 weeks. No significant changes were observed between control and krill powder groups ($p < 0.05$).

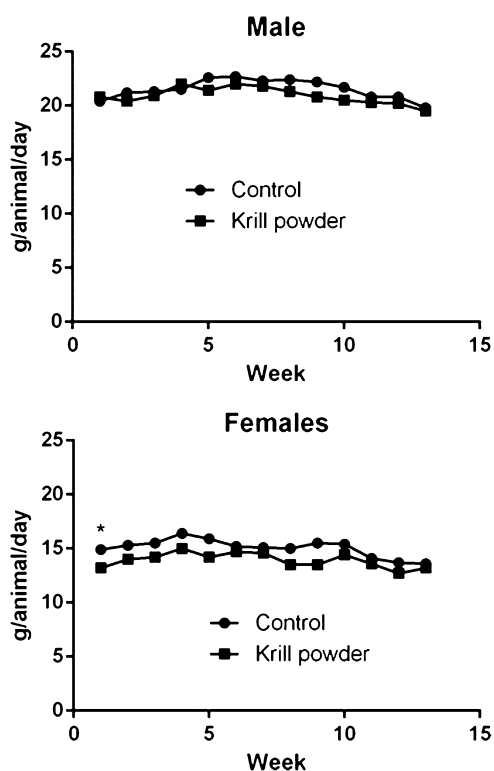


Fig. 2. Group mean food consumption of male and female Han Wistar rats fed diets containing krill powder for 13 weeks. Control and krill powder groups were not statistically different, except for females consumption at Week 1 ($p < 0.05$).

Table 2
Haematology and coagulation at termination of the study.

	Control	Krill powder
Males		
Haemoglobin (g/dL)	15.7 ± 0.8	15.5 ± 0.4
Red blood cells ($\times 10^{12}/L$)	8.55 ± 0.70	8.49 ± 0.29
Haematocrit (L/L)	0.43 ± 0.02	0.43 ± 0.01
Mean cell haemoglobin (pg)	18.5 ± 1.5	18.3 ± 0.8
Mean cell volume (fL)	50.6 ± 3.2	50.8 ± 1.5
Mean cell haemoglobin concentration (g/dL)	36.5 ± 0.9	36.0 ± 1.2
Reticulocytes (%)	1.8 ± 0.3	2.1 ± 0.2
White blood cells ($\times 10^9/L$)	6.41 ± 1.27	6.57 ± 1.31
Neutrophils ($\times 10^9/L$)	1.12 ± 0.37	1.00 ± 0.21
Lymphocytes ($\times 10^9/L$)	5.00 ± 1.04	5.27 ± 1.16
Monocytes ($\times 10^9/L$)	0.10 ± 0.02	0.12 ± 0.03
Eosinophils ($\times 10^9/L$)	0.14 ± 0.05	0.14 ± 0.04
Basophils ($\times 10^9/L$)	0.02 ± 0.01	0.01 ± 0.01
Large unclassified cells ($\times 10^9/L$)	0.04 ± 0.01	0.04 ± 0.01
Platelets ($\times 10^9/L$)	747 ± 119	702 ± 182
Prothrombin time (s)	15 ± 1	16 ± 1
Activated partial thromboplastin time (s)	20 ± 2	22 ± 2
Females		
Haemoglobin (g/dL)	15.0 ± 0.6	14.4 ± 0.7
Red blood cells ($\times 10^{12}/L$)	8.06 ± 0.45	7.80 ± 0.41
Haematocrit (L/L)	0.42 ± 0.02	0.40 ± 0.02
Mean cell haemoglobin (pg)	18.6 ± 0.8	18.5 ± 0.4
Mean cell volume (fL)	51.5 ± 1.8	51.2 ± 0.6
Mean cell haemoglobin concentration (g/dL)	36.1 ± 0.9	36.1 ± 0.8
Reticulocytes (%)	2.6 ± 0.5	2.4 ± 0.8
White blood cells ($\times 10^9/L$)	4.54 ± 0.56	4.81 ± 0.96
Neutrophils ($\times 10^9/L$)	0.62 ± 0.2	0.70 ± 0.37
Lymphocytes ($\times 10^9/L$)	3.71 ± 0.65	3.88 ± 0.90
Monocytes ($\times 10^9/L$)	0.08 ± 0.02	0.10 ± 0.04
Eosinophils ($\times 10^9/L$)	0.09 ± 0.03	0.09 ± 0.04
Basophils ($\times 10^9/L$)	0.01 ± 0.01	0.01 ± 0.01
Large unclassified cells ($\times 10^9/L$)	0.03 ± 0.02	0.03 ± 0.01
Platelets ($\times 10^9/L$)	692 ± 108	715 ± 81
Prothrombin time (s)	15 ± 1	14 ± 1
Activated partial thromboplastin time (s)	21 ± 2	22 ± 3

No significant changes were observed between the control and krill powder group ($p < 0.05$). All values are means \pm SD for 6–10 animals.

Tables 2 and 3). Visual inspection of water bottles did not show any differences between the groups throughout the treatment period.

3.3. Haematology, clinical chemistry and urinalysis

Haematology values at the termination of the study are presented in Table 2. There were no differences in any of the parameters that were considered to be due to the consumption of krill powder. There were, however, some significant changes in clinical chemistry measurements (Table 3). Total protein was increased significantly in both males and females fed the krill powder diets. Globulin levels in the krill powder fed animals were also significantly increased in both sexes, compared to control. This led to a decrease in the albumin:globulin ratio, but in male animals only. The fourth statistically different observation was an

Table 3
Clinical chemistry at termination of the study.

	Control	Krill powder
Males		
Alkaline phosphatase (IU/L)	97 ± 17	104 ± 22
Alanine aminotransferase (IU/L)	23 ± 5	28 ± 4
Aspartate aminotransferase (IU/L)	60 ± 8	60 ± 4
Lactate dehydrogenase (IU/L)	102 ± 27	112 ± 41
Urea (mmol/L)	5.7 ± 0.3	5.8 ± 0.5
Glucose (mmol/L)	11.94 ± 1.57	12.07 ± 1.09
Total bilirubin ($\mu\text{mol/L}$)	1.7 ± 0.0	1.7 ± 0.0
Cholesterol (mmol/L)	1.9 ± 0.2	1.8 ± 0.3
Triglycerides (mmol/L)	2.53 ± 0.60	2.57 ± 0.94
Total protein (g/L)	66 ± 2	69 ± 3 ^a
Albumin (g/L)	42 ± 1	42 ± 1
Globulin (g/L)	24 ± 2	27 ± 2 ^a
Albumin:globulin ratio	1.7 ± 0.1	1.6 ± 0.1 ^a
Sodium (mmol/L)	142 ± 3	143 ± 1
Potassium (mmol/L)	4.2 ± 0.2	4.3 ± 0.2
Phosphate (mmol/L)	1.24 ± 0.27	1.45 ± 0.13
Calcium (mmol/L)	2.68 ± 0.04	2.70 ± 0.09
Females		
Alkaline phosphatase (IU/L)	111 ± 76	58 ± 23
Alanine aminotransferase (IU/L)	19 ± 4	21 ± 4
Aspartate aminotransferase (IU/L)	60 ± 8	61 ± 11
Lactate dehydrogenase (IU/L)	73 ± 10	158 ± 202
Urea (mmol/L)	7 ± 0.9	5.8 ± 0.6
Glucose (mmol/L)	10.75 ± 1.48	9.26 ± 1.20
Total bilirubin ($\mu\text{mol/L}$)	1.9 ± 0.5	1.7 ± 0.0
Cholesterol (mmol/L)	1.2 ± 0.3	1.2 ± 0.3
Triglycerides (mmol/L)	1.39 ± 0.78	1.70 ± 0.76
Total protein (g/L)	69 ± 3	73 ± 4 ^a
Albumin (g/L)	49 ± 3	51 ± 4
Globulin (g/L)	19 ± 2	22 ± 2 ^b
Albumin:globulin ratio	2.6 ± 0.3	2.4 ± 0.3
Sodium (mmol/L)	141 ± 1	142 ± 1
Potassium (mmol/L)	3.6 ± 0.2	3.9 ± 0.3 ^a
Phosphate (mmol/L)	1.12 ± 0.24	1.26 ± 0.23
Calcium (mmol/L)	2.68 ± 0.04	2.70 ± 0.08

All values are means \pm SD for 8–10 animals.

^a $p < 0.05$ (significantly different from control).

^b $p < 0.01$ (significantly different from control).

increase in potassium level in female rats fed the krill powder diet. No differences in urinalysis parameters that were considered to be related to the consumption of the krill powder diet in either of the sexes were seen (Table 4).

Table 4
Urinalysis at termination of the study.

	Control	Krill powder
Males		
Urine specific gravity	1.055 ± 0.012	1.064 ± 0.011
Urine volume (mL)	0.7 ± 0.5	0.4 ± 0.3
pH	8.0 ± 1.1	7.3 ± 1.1
Females		
Urine specific gravity	1.058 ± (–)	1.052 ± 0.000
Urine volume (mL)	0.1 ± 0.0	0.2 ± 0.2
pH	6.6 ± 0.5	7.3 ± 1.2

No significant changes were observed between the control and krill powder groups ($p < 0.05$). All values are means \pm SD. The number of samples in males was: urine specific gravity: $n = 7-9$; urine volume: $n = 9$; pH: $n = 8-9$. The number of samples in females was: urine specific gravity: $n = 1-2$; urine volume: $n = 6-10$; pH: $n = 4-5$. (–) Due to the small number of female samples, an accurate comparison could not be made against the control group, although no numerical differences were noted.

Table 5
Relative organ weights (% of body weight).

	Control	Krill powder
Males		
Adrenals	0.013 ± 0.002	0.012 ± 0.001
Brain	0.43 ± 0.04	0.42 ± 0.04
Epididymides	0.29 ± 0.05	0.28 ± 0.04
Heart	0.29 ± 0.02	0.27 ± 0.02
Kidneys	0.61 ± 0.02	0.60 ± 0.06
Liver	3.3 ± 0.2	3.6 ± 0.4
Lung	0.38 ± 0.04	0.40 ± 0.05
Pituitary	0.0018 ± 0.0003	0.0024 ± 0.0008
Prostate	0.10 ± 0.02	0.11 ± 0.02
Spleen	0.15 ± 0.02	0.15 ± 0.01
Testes	0.76 ± 0.09	0.76 ± 0.10
Thymus	0.11 ± 0.02	0.12 ± 0.04
Thyroid	0.0057 ± 0.0012	0.0057 ± 0.0018
Females		
Adrenals	0.029 ± 0.007	0.029 ± 0.007
Brain	0.70 ± 0.09	0.73 ± 0.06
Heart	0.34 ± 0.04	0.33 ± 0.03
Kidneys	0.66 ± 0.08	0.69 ± 0.05
Liver	3.2 ± 0.3	3.6 ± 0.4
Lung	0.54 ± 0.09	0.54 ± 0.07
Ovaries	0.042 ± 0.008	0.043 ± 0.007
Pituitary	0.0045 ± 0.0007	0.0049 ± 0.0009
Spleen	0.21 ± 0.02	0.23 ± 0.02
Thymus	0.17 ± 0.04	0.15 ± 0.01
Thyroid	0.0075 ± 0.0016	0.0076 ± 0.0014
Uterus	0.25 ± 0.16	0.24 ± 0.06

No significant changes were observed between the control and krill powder groups ($p < 0.05$). All values are means ± SD for 8–10 animals.

3.4. Neurotoxicity, neurobehavioral studies and ophthalmoscopy

There were no abnormalities detected in the brain in any animal after histological evaluation at the termination of the study. Moreover, there were no test material-related changes in motor activity for either sex. Likewise, krill powder administration did not affect any of the parameters measured by a functional observation battery of assessments that included landing foot splay, fore grip, hind grip and tail flick. There were no ophthalmoscopy findings that were considered to be related to administration of krill powder for 13 weeks.

3.5. Pathology

Both relative (Table 5) and absolute (Table 6) organ weights are presented. We observed a decreased absolute heart weight in both sexes, compared to control animals. However, after adjustment for body weight, no significant changes in heart weights were observed.

After krill powder administration for 13 weeks, prominent liver lobulation was observed in 4 out of 10 males, but not in any of the female animals (Table 7). Periportal microvesicular hepatocyte vacuolation was observed in 2 out of 10 males which received the krill powder diet, but this finding was not statistically different, when compared with the control animals ($p < 0.05$). This correlated with the findings of prominent liver lobulation in 4 male animals observed at necropsy. Since the hepatocyte vacuolation in the two male animals was not associated with

Table 6
Absolute organ weights (g).

	Control	Krill powder
Males		
Final body weight	481 ± 51	477 ± 23
Adrenals	0.062 ± 0.007	0.056 ± 0.008
Brain	2.05 ± 0.05	1.96 ± 0.05
Epididymides	1.36 ± 0.17	1.35 ± 0.19
Heart	1.36 ± 0.13	1.26 ± 0.09 ^a
Kidneys	2.95 ± 0.34	2.86 ± 0.33
Liver	15.96 ± 1.95	17.30 ± 2.52
Lung	1.82 ± 0.17	1.87 ± 0.27
Pituitary	0.009 ± 0.001	0.012 ± 0.004
Prostate	0.49 ± 0.1	0.54 ± 0.09
Spleen	0.69 ± 0.05	0.70 ± 0.07
Testes	3.62 ± 0.27	3.59 ± 0.46
Thymus	0.54 ± 0.09	0.51 ± 0.13
Thyroid	0.027 ± 0.005	0.027 ± 0.009
Females		
Final body weight	263 ± 38	247 ± 16
Adrenals	0.076 ± 0.016	0.073 ± 0.016
Brain	1.82 ± 0.08	1.79 ± 0.14
Heart	0.90 ± 0.12	0.82 ± 0.07 ^a
Kidneys	1.71 ± 0.2	1.69 ± 0.12
Liver	8.43 ± 1.01	8.77 ± 1.06
Lung	1.40 ± 0.24	1.32 ± 0.17
Ovaries	0.11 ± 0.02	0.11 ± 0.02
Pituitary	0.012 ± 0.002	0.012 ± 0.002
Spleen	0.55 ± 0.08	0.56 ± 0.05
Thymus	0.44 ± 0.12	0.36 ± 0.05
Thyroid	0.020 ± 0.005	0.019 ± 0.003
Uterus	0.65 ± 0.44	0.58 ± 0.17

All values are means ± SD for 8–10 animals.

^a $p < 0.05$ (significantly different from control).

Table 7
Necropsy and histological findings.

	Control	Krill powder
Males (n = 10)		
Liver lobulation	0	4
Hepatocyte vacuolation	0	2
Females (n = 10)		
Liver lobulation	0	0
Hepatocyte vacuolation	0	0

Necropsy (prominent liver lobulation) and histopathology (periportal microvesicular hepatocyte vacuolation) was done in 10 male and female animals after 13 weeks of control or krill powder treatment.

hepatocellular necrosis or inflammation, and clinical pathology findings suggesting liver impairment was not detected, this finding was considered adaptive and non-adverse. There were no liver vacuolation observations in females receiving krill powder or in control animals.

4. Discussion

This 13-week subchronic toxicity study in rats using krill powder at a dose of 9.67% in the diet demonstrates a lack of toxicologically significant adverse effects and demonstrates that krill powder is a safe source of omega-3 fatty acids. The 9.67% dose of krill powder was chosen since it corresponds to a dose of 5% krill oil, which was previously found to be the NOAEL in a 13-week toxicity study [22]. Given that the effects of the toxicity study were not adverse in nature, the NOAEL for the conditions of this study was

considered to be 9.67% krill powder (equating to 5357 mg krill powder/kg body weight/day for males and 6284 mg krill powder/kg body weight/day for females). However, since only one dose of krill powder was used in this study, no definitive statement on NOAEL can be made, which should be seen as a limitation to this study.

No differences were noted in body weight or food consumption during the krill powder treatment. Administration of krill powder resulted in abnormally pale and/or yellow coloured faeces, which was considered a result of the test diet which itself had a red colour due to the astaxanthin content in krill powder. One animal in the krill powder group was euthanized during the study due to an open and wet lesion on dorsal neck. Even if no significant alterations was observed in haematology parameters, some differences in clinical chemistry values (total protein, globulin and potassium levels) were noted in the krill powder-treated group, when compared to control. However, the findings were considered to be of no toxicological significance since the changes were small and not related to histopathological changes.

Hepatocyte vacuolation was observed in two male rats fed krill powder after microscopic evaluation. This might be due to an accumulation of triglycerides in the liver due to the high dose of lipids given [23]. Such observations has been seen in other studies and is considered to be a compensatory transient process [24].

Significantly decreased absolute heart weights for both male and female animals receiving krill powder was observed in the study. In a previous study with Zucker rats, a decreased amount of fat in the heart after krill oil treatment was observed [11]. The decreased heart weight observed in the current study could possibly be explained by similar fat-lowering mechanisms. However, when evaluated relative to body weight, the heart weight was not significantly altered in the krill powder animals, when compared to the control group.

In conclusion, krill powder demonstrated no adverse toxicological in-life, haematology or clinical chemistry effects at an inclusion level of 9.67% in diets for rats, when given for 13 weeks. The negative findings were restricted to hepatocyte vacuolation in male animals with no accompanying increase in liver weight.

Conflict of interest

Kjetil Berge and Lena Burri are employees of Aker BioMarine Antarctic AS.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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Contributions: KB and BR designed the study. BR contributed to the performance of the trial. BR, KB and LB interpreted the data and wrote the paper. All authors read and approved the final manuscript.

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