IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re *Inter Partes* Reexamination of U.S. Patent No. 8,030,348

Entitled: NATURAL MARINE SOURCE PHOSPHOLIPIDS COMPRISING POLYUNSATURATED FATTY ACIDS AND THEIR APPLICATIONS

Issued: 4 October 2011 to Sampalis

Declaration by Bjørn Ole Haugsgjerd, MSc, in Support of Request for Inter Partes Reexamination of U.S. Patent NO. 8,030,348

EFS WEB Filed

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Commissioner for Patents
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I, Bjørn Ole Haugsgjerd, MSc, state as follows:

1. My present position is Deputy manager at Nofima BioLab, Norway

2. At the request of Aker Biomarine ASA, I have extracted lipid fractions from *Euphausia superba* and *Euphausia pacifica* by the methods described in Beaudoin I (WO 00/23546), Beaudoin II (Canadian Application 2,251,265) and Maruyama (Japanese Laid Open Application 2909508). Following the extraction, I shipped the samples to Vitas AS, Oslo, Norway, for analytical analysis. Frozen *Euphausia superba* was provided by Aker Biomarine ASA. Frozen *Euphausia pacifica* was purchased from Fish and Fins Limited, East Sussex, UK.

- Extract with acetone at 4C at a sample:acetone ratio of 1:6 (w/v) for 2 hours with 20 minutes of swirling.
- Filter on organic solvent resistant filter paper under reduced pressure at 4C.
- Wash solid material on filter with a sample:acetone ratio of 1:2 (w/v) with pure and cold acetone.
- Combine filtrates, mark with Fraction I - Acetone extract E. pacifica (or superba) and store at -20C.
- Divide solid material on filter into two aliquots, aliquot 1 and aliquot 2.
- Extract aliquot 1 with pure ethanol at sample:ethanol ratio of 1:2 (w/v) for 30 minutes at 4C.
- Filter on organic solvent resistant filter paper under reduced pressure at 4C.
- Evaporate solvent under reduced pressure to provide Fraction IIa.
- Extract aliquot 2 with pure ethyl acetate at sample:ethyl acetate ratio of 1:2 (w/v) for 30 minutes at 4C.
- Filter on organic solvent resistant filter paper under reduced pressure at 4C.
- Evaporate solvent under reduced pressure to provide Fraction IIb.

IIa	70	5	E. pacifica (or superba) Fraction IIa 70 degr 5 min
IIa	125	15	E. pacifica (or superba) Fraction IIa 125 degr 15 min
IIb	-	-	E. pacifica (or superba) Fraction IIb not heated
IIb	70	5	E. pacifica (or superba) Fraction IIb 70 degr 5 min
IIb	125	15	E. pacifica (or superba) Fraction IIb 125 degr 15 min

- Store all samples at -20C until further analysis
- Heat treatments to be conducted at Vitas AS according to the table above before analysis.

- Vacuum freeze dry krill until water content is 6% or less.
- Homogenize freeze-dried krill with 100% ethanol (20 parts ethanol/1 part krill, w/w) until total lipids are extracted.
- Remove ethanol by evaporation under reduced pressure.
- Re-extract with 10 parts ethanol w/w.
- Remove ethanol by evaporation under reduced pressure.
- Save a sample of the ethanol–extracted lipids for analysis. Label *E. superba* or *E. pacifica* ETOH extract. Flush samples with nitrogen gas before sealing to provide an inert atmosphere. Store at -20C until further analysis.
- Dissolve ethanol extracted lipids in 20 parts acetone to separate into soluble and insoluble fraction.
- Separate insoluble fraction by centrifugation at 2000 rpm for 5 minutes and decant off the acetone soluble fraction. Repeat wash and centrifugation with acetone twice.
- Remove acetone by evaporation under reduced pressure to provide a crude phospholipid extract. Save a sample of the crude phospholipid extract for analysis. Label E. superba or E. pacifica crude PL extract. Flush samples with nitrogen gas before sealing to provide an inert atmosphere. Store at -20C until further analysis.

Respectfully submitted,

Bjørn Ole Haugsgjerd — 4. oktober 2011

Bjørn Ole Haugsgjerd, MSc — Date