

**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

Bjørn Ole Haugsgjerd, MSc

4. October 2011

Date

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