Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Risk assessment regarding processing requirements of by-products from aquaculture for use in fish feed

Opinion of the Panel on Animal Feed of the Norwegian Scientific Committee for Food Safety

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The Panel on Animal Feed (Panel 6) established the following *ad hoc* group consisting of:

Live L. Nesse (Chair)
Espen Rimstad
Einar Ringø
Jan Thomas Rosnes

Contributors

Persons working for VKM, either as appointed members of the Committee or as ad hoc experts, do this by virtue of their scientific expertise, not as representatives for their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed an ad hoc group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority. The members of the ad hoc group are acknowledged for their valuable work on this opinion.

The members of the ad hoc group are:

VKM members

Live L. Nesse (Chair), Panel on Animal Feed

Espen Rimstad, Panel on Animal Health and Welfare

Einar Ringø, Panel on Animal Feed

External experts

Jan Thomas Rosnes, Nofima

VKM would like to acknowledge Tor Atle Mo at Norwegian Veterinary Institute, for his contribution to the chapter about parasites.

Assessed by

The report from the *ad hoc* group has been evaluated and approved by the Panel on Animal Feed of VKM.

Panel on Animal Feed:

Aksel Bernhoft (Chair), Live L. Nesse, Birger Svihus, Einar Ringø, Jan Raa, Marit Aursand, Bente Torstensen, Robin Ørnsrud

Scientific coordinator from the secretariat: Tron Øystein Gifstad

1. Summary

1.1. Background

The Norwegian Food Safety Authority (NFSA) wants to ensure that fish meal and oil from clinically healthy farmed fish does not constitute a risk of infection if it is fed to farmed fish of other species

NFSA has therefore requested that the Norwegian Scientific Committee for Food Safety (VKM) conduct a risk assessment regarding effects on fish health caused by the use of by-products from farmed fish in the feed for other species of farmed fish.

Processing of by-products is regulated by the provisions of the by-products Regulation (EC) 1774/2002.

Today there are discrepancies between the processing requirements recommended as safe by the NFSA for both silage and fresh by-products from farmed fish, particle size ≤ 10 mm, heating ≥ 85°C for ≥ 25 minutes, and particle size ≤ 10 mm, heating ≥ 76°C for ≥ 20 minutes, as the industry has requested to use for fresh (not silage) raw materials from aquaculture.

NFSA has asked VKM to assess the risk to fish health by the use of untreated animal by-products (ABP) of category 3 (by-products from fish for human consumption), as well as ABP category 3 materials, and fishmeal and fish oil originating from such material following heat treatment at 76°C for 20 minutes or 85°C for 25 minutes. NFSA has asked specifically about the risks of using processed salmon oil in salmon feed. Furthermore NFSA has asked to what extent silaging (pH equal to 4 or below) of by-products will affect the outcome of the two heat treatment regimes. Finally, NSFA wanted to know if the infectious pancreatic necrosis (IPN) virus is a suitable indicator for sufficient inactivation of fish pathogens.

An *ad hoc* group was appointed to perform this task, and the Animal Feed Panel, hereafter referred to as the Panel, has approved the report.

1.2. Presumptions

The Panel has no knowledge of any data regarding the effect of heat inactivation or acidification of Piscine reovirus (PRV). Lacking this information, the Panel has chosen to anticipate that the PRV is not more heat and acid resistant than IPN virus, which is the most resistant of those viruses that have been assessed. Likewise, information on heat and acid tolerance is also very limited or missing for some of the bacteria assessed. Based on general information on psychrotrophic bacteria, the Panel has chosen to anticipate that these bacteria are less heat and acid resistant than *Renibacterium salmoninarum* and *Lactococcus garvieae*, which are the most resistant of those assessed. These presumptions may prove to be wrong when relevant information becomes available, and this uncertainty should be kept in mind when reading the Panel's conclusions.

When assessing fish oil and fishmeal, it is presumed that the oil and meal has not been exposed to heat treatment with higher temperatures earlier in the production process.

1.3. Untreated ABP category 3 material

The Panel concludes that the probability from exposure in untreated ABP category 3 material may be very high for Piscine reovirus (PRV) and moderate to high for infectious pancreatic necrosis (IPN) virus *Francisella* sp., *Vibrio(Listonella) anguillarum* and *Lepeophtheirus salmonis* (Salmon louse). The probability is moderate for Salmon pancreas disease virus (SPDV) in endemic area, low to moderate for *Flavobacterium phsychrophilum*, and very low to low for Infectious salmon anemia virus, Nodavirus, SPDV outside endemic area,

Renibacterium salmoninarum, Aeromonas salmonicida subsp. *salmonicida* and *Yesinia ruckeri.* The probability of exposure to Infectious hematopoietic necrosis virus (IHNV), Viral hemorrhagic septicemia virus (VHSV)*, Lactococcus garviae* and *Gyrodactylus salaris* is negligible. For each pathogen, the probability relates to the ABP category 3 material from the susceptible species.

1.4. Heat treatment at 76°C for 20 minutes

After heat treatment of ABP category 3 materials at 76 °C for 20 minutes, the probability of infectious pancreatic necrosis (IPN) virus, and possibly also PRV, being present is considered to be low. The Panel cannot exclude the possibility that that the fraction of viruses surviving a suboptimal heat treatment may be more heat resistant than the majority of the virus population. If so, the probability may increase over time if these viruses are recirculated back to the fish farm environment. The probability of presence of all other organisms included in this assessment is considered to be negligible.

After heat treatment of **fishmeal** originating from such materials, the probability is the same as after treatment of ABP category 3 materials for the viruses and parasites assessed. After heat treatment of **fish oil** the probability is very low for (IPN) virus, and possibly also PRV, and negligible for all other viruses and parasites assessed. However, the Panel does not have sufficient information to anticipate the probability of presence of bacteria after heat treatment of fish oil and fishmeal at 76°C for 20 minutes.

1.5. Heat treatment at 85°C for 25 minutes

Following heat treatment of ABP category 3 materials and fishmeal originating from such material at 85°C for 25 minutes, the Panel anticipates that the probability of presence of all viruses, bacteria and parasites assessed is negligible.

After heat treatment of fish oil originating from such materials, the probability is negligible for the viruses and parasites assessed. However, the Panel does not have sufficient information to anticipate the probability of presence of bacteria in fish oil after heat treatment at 85°C for 25 minutes.

1.6. Silaging at pH 4 or below

The Panel anticipates that silaging (pH equal to 4 or below obtained by organic acid) of ABP category 3 materials will inactivate IHNV, ISAV, most of the bacteria and the parasites, but have little or no effect on *Lactococcus garvieae* and the other viruses.

1.7. Using processed salmon oil in salmon feed

Heat treatment of fish oil at 76°C for 20 minutes will not reduce the probability of IPN virus, and possibly also PRV, to negligible. IPN virus can infect all farmed fish species in Norway, even though the infection dose may be low. PRV can infect salmonids.

The probability of presence of all viruses and parasites assessed after heat treatment of fish oil at 85°C for 25 minutes is negligible. The Panel does not have sufficient information to anticipate the probability of presence of bacteria.

1.8. IPN virus a suitable indicator for sufficient inactivation of fish pathogens

The IPN virus appears to be more heat resistant than the other fish pathogens assessed. This should make IPN virus a suitable indicator for testing methods for heat inactivation. However, the Panel is of the opinion that methods more suitable for the detection of viable IPN virus

should be established. Furthermore, the Panel suggests that NFSA also considers using a bacterial indicator. Bacteria have several stress protection mechanisms not shared by virus. Consequently, a virus may not be a representative indicator for bacteria in all test situations.

2. Norsk sammendrag

2.1. Bakgrunn

Mattilsynet ønsker å sikre at prosessering av mel og olje fra biprodukter fra klinisk frisk oppdrettsfisk, ikke skal innebære risiko for smitte ved bruk som fôrmiddel i fôr til annen oppdrettsfisk.

Mattilsynet har derfor bedt Vitenskapskomiteen for mattrygghet (VKM) om en risikovurdering av fiskehelsen ved bruk av biprodukter fra oppdrettsfisk i fôr til annen oppdrettsfisk, som ikke er av samme art.

Behandling av biprodukter reguleres av bestemmelsene av biproduktforordningen (EC) 1774/2002.

I dag er at det er sprik mellom det prosesseringskravet Mattilsynet anbefaler som trygt for både ensilerte og ferske biprodukter fra oppdrettsfisk; partikkelstørrelse ≤10mm, oppvarming ≥ 85 °C i ≥25 min), og ønsket fra næringen om å senke temperaturkravet i ferskt (ikke ensilert) råstoff fra oppdrettsnæringen til ≥ 76 °C i ≥20 min.

Mattilsynet har bedt VKM å vurdere risikoen for fiskehelsen ved bruk av ubehandlede biprodukter fra dyr (ABP) av kategori 3 (biprodukter fra fisk til humant konsum), samt ABP kategori 3-materialer, og fiskemel og fiskeolje som stammer fra slikt materiale med påfølgende varmebehandling ved 76°C i 20 minutter eller 85°C i 25 minutter. Mattilsynet spør spesielt om risikoen ved å bruke prosessert lakseolje i laksefôr. Videre spør Mattilsynet om i hvilken grad ensilering (pH lik 4 eller mindre) av biprodukter vil påvirke utfallet av ulike varmebehandlingsregimer. Til slutt ønsker Mattilsynet å vite om Infeksiøs pankreasnekrose (IPN)-viruset er en egnet indikator for å vise tilstrekkelig deaktivering av fiskepatogener.

En *ad hoc*-gruppe ble oppnevnt for å arbeide med dette oppdraget, og faggruppen for fôr til terrestriske og akvatiske dyr, heretter kalt faggruppen, har godkjent rapporten.

2.2. Forutsetninger

Faggruppen har ikke kjennskap til data angående effekten av varme- eller syreinaktivering av Piscint reovirus (PRV). Siden denne informasjonen mangler, har faggruppen valgt å forutsette at PRV ikke er mer motstandsdyktige mot varme og syre enn IPN-virus, som er den mest motstandsdyktige av de agens som har blitt vurdert. Tilsvarende er informasjonen om varme- og syretoleranse også svært begrenset eller mangler helt for noen av bakterieartene som er vurdert. Basert på generell informasjon om bakterier som gir sykdom hos oppdrettsfisk har faggruppen valgt å forutsette at de er mindre motstandsdyktige mot varme og syre enn *Renibacterium salmoninarum* og *Lactococcus garvieae*, som er de mest resistente bakterieartene av de som har blitt vurdert. Disse forutsetningene kan vise seg å være feil når relevant informasjon blir tilgjengelig, og denne usikkerheten bør tas i betraktning når man leser konklusjonene fra faggruppen.

Ved vurdering av varmebehandling av fiskeolje og fiskemel er det forutsatt at materialet ikke er behandlet med høyere temperaturer tidligere i produksjonsprosessen.

2.3. Ubehandlet ABP kategori 3-materiale

Faggruppen konkluderer med at sannsynligheten for tilstedeværelse i ubehandlet ABP kategori 3-materiale kan være svært høy for PRV og moderat til høy for IPN-virus, *Vibrio (Listonella) anguillarum, Francisella* sp. og *Lepeophtheirus salmonis* (lakselus). Sannsynligheten er moderat for Salmon pancreas disease virus (SPDV, også kalt Salmonid alphavirus (SAV)) i endemisk område, lav til moderat for *Flavobacterium phsychrophilum,* og svært lav til lav for Infeksiøs lakseanemi virus, Nodavirus, SPDV utenfor endemisk område, *Renibacterium salmoninarum, Aeromonas salmonicida* subsp. *salmonicida* og *Yesinia ruckeri.* Sannsynligheten for eksponering for Infectious hematopoietic necrosis virus (IHNV), Viral hemorrhagic-septicemia virus (VHSV), *Lactococcus garviae* og *Gyrodactylus salaris* er ubetydelig.

For hvert agens er sannsynligheten knyttet til ABP og kategori 3-materiale fra mottagelige arter.

2.4. Varmebehandling ved 76°C i 20 minutter

Etter varmebehandling av ABP kategori 3-materialet ved 76°C i 20 minutter anses sannsynligheten for at IPN-virus, og muligens også PRV, skal være tilstede som lav. Faggruppen kan ikke utelukke at fraksjonen av virus som overlever en suboptimal varmebehandling kan være mer varmeresistent enn majoriteten av viruspopulasjonen. I så fall kan sannsynligheten øke over tid hvis disse virusene blir resirkulert tilbake til miljøet i oppdrettsanlegg. Sannsynligheten for forekomst av alle andre virus, alle bakterier og alle parasitter som er inkludert i denne vurderingen, anses å være ubetydelig.

For virus og parasitter er sannsynligheten den samme etter varmebehandling av ABP kategori 3-materiale som for **fiskemel** som stammer fra slike materialer. Etter varmebehandling av **fiskeolje** er sannsynligheten svært lav for virus (IPN) og muligens også PRV, og ubetydelig for alle andre virus og parasitter som er vurdert. Faggruppen har imidlertid ikke nok informasjon til å forutsi sannsynligheten for tilstedeværelse av bakterier etter varmebehandling av fiskeolje og fiskemel ved 76°C i 20 minutter.

2.5. Varmebehandling ved 85 °C i 25 minutter

Etter varmebehandling av ABP kategori 3-materiale og fiskemel som stammer fra slikt materiale ved 85°C i 25 minutter, forventer faggruppen at sannsynligheten er ubetydelig for tilstedeværelse av alle virus, bakterier og parasitter som er vurdert.

Etter varmebehandling av fiskeolje og fiskemel som stammer fra slikt materiale, er sannsynligheten ubetydelig for de virus og parasitter som er vurdert. Faggruppen har imidlertid ikke nok informasjon til å forutsi sannsynligheten for tilstedeværelsen av bakterier etter varmebehandling av fiskeolje ved 85°C i 25 minutter.

2.6. Ensilering ved pH 4 eller lavere

Faggruppen forventer at ensilering (pH lik 4 eller lavere ved bruk av annen organisk syre) av ABP kategori 3-materiale vil inaktivere IHNV, ISAV, og de fleste bakterier og parasitter, men ha liten eller ingen effekt på *Lactococcus garvieae* og de andre virusene.

2.7. Bruk av behandlet lakseolje i lakseför

Varmebehandling av fiskeolje ved 76°C i 20 minutter vil ikke redusere sannsynligheten for IPN- virus, og muligens heller ikke for PRV, til ubetydelig. IPN-virus kan infisere alle oppdrettsfiskearter i Norge, selv om mengden av virus som gir infeksjon kan være lav.

Sannsynligheten for tilstedeværelse er ubetydelig for alle vurderte virus og parasitter etter varmebehandling av fiskeolje ved 85°C i 25 minutter. Faggruppen har ikke tilstrekkelig informasjon til å forutsi sannsynligheten for tilstedeværelsen av bakterier.

2.8. IPN-virus som en egnet indikator for tilstrekkelig inaktivering av fiskepatogener

IPN-viruset synes å være mer varmemotstandsdyktig enn de andre fiskepatogenene som er vurdert. Dette bør gjøre IPN-virus til en egnet indikator for testing av metoder for varmeinaktivering. Faggruppen anbefaler at det etableres en bedre egnet metode for å påvise virus. Videre foreslår faggruppen at Mattilsynet også bør vurdere å bruke en bakteriell indikator. Bakterier har flere stressbeskyttelsemekanismer som virus ikke har. Et virus vil derfor ikke nødvendigvis være en representativ indikator for bakterier i alle testsituasjoner.

3. Abbreviations and definitions

Abbreviation	Definition
ABP	Animal by-products
By-products of category 3	By-products from fish for human consumption, including by-products from fish farms with disease restrictions (listed diseases) where the fish are not clinically ill
cfu	Colony forming unit (unit for enumeration of bacteria)
D value	The heating time that results in 90% destruction of the existing microbial population.
FSPM	Fish silage processing method
IHNV	Infectious hematopoietic necrosis virus
IPN virus	Infectious pancreatic necrosis virus
ISAV	Infectious salmon anaemia virus
NFSA	Norwegian Food Safety Authority (Mattilsynet)
NVI	Norwegian Veterinary Institute
PRV	Piscine reovirus
pfu	Plack forming unit (unit for enumeration of viruses)
Psychrotrophic microorganism	A microorganism that can survive and grow at low temperatures, but grows optimally between 15 and 20 °C.
SPDV (SAV)	Salmon pancreas disease virus also called Salmonoid alpha virus
$TCID_{50}$	50% Tissue Culture Infective Dose. The amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated. Expressed as $TCID_{50}$/ml.
VHSV	Viral hemorrhagic septicaemia virus
VKM	Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet)
Z-value	Temperature coefficient, i.e. the number of degrees of temperature (°C) necessary to change the D-value with one logarithmic unit

4. Background

In Norway traditionally fishmeal has been produced from wild caught fish. The large salmon production in Norway generates about 195 000 tons residual raw materials per year (Rubin 2009). This raw material can either be handled according to hygiene legislation for human consumption, or defined as a by-product, according to the by-product legislation, and used as feed. This is valuable marine raw material that could represent about 65-70000 tons of fishmeal / fish oil (statement from the industry).

Processing of by-products is regulated by the provisions of the by-products Regulation (EC) 1774/2002. This Regulation specifies different methods for handling by-products, but none of these secure the fish health.

From March 2011, the new by-product Regulations (1069/2002 and 142/2011), were implemented in the EU. These new regulations will also be implemented in Norway, but it is not clear when the regulations will come into force in Norway.

Animal by-products from fish farms are allowed to be used in feed for farmed fish, however, the use of the products are restricted by the species barrier. Species restrictions are related to the fishmeal (protein fraction), and as an example, it is not allowed to feed salmon meal to salmon. Fishmeal from salmon can be fed to other fish species. Pure fish oil (protein free) has no species restrictions related to the application. Animal by-products from fish farms are also allowed to be used in feed for fur animals.

EU's new by-product regulation has emphasized the requirements for microbiological safety of fishmeal from aquaculture animals, and also the restrictions related to species barrier, are maintained.

4.1. Raw materials

Raw materials that are allowed to be used in the production of fishmeal are defined as category 3 in the by-product regulation.

By-products from fish for human consumption are category 3 materials, and can be processed to feed for food producing animals (non-ruminants). Fish that died for other reason than being used for human consumption are category 2 material, and cannot be used as feed.

By-products from farms with disease restrictions (listed diseases) where the fish are not clinically ill, is also considered to be category 3 material. As far as the fish can be used for human consumption, the by-products are considered category 3. This means that the fishmeal produced from category 3 materials from farms with disease restrictions may also be used in feed.

The amount of infectious substances of category 3 material from farmed fish is difficult to predict, because clinically healthy fish may also carry high levels of infectious substances. It is therefore of great importance that the processing method used in fishmeal and fish oil production, must be sufficient for inactivating all types of infectious agents that can occur in the aquaculture industry.

If the processing method can provide documentation that all known fish pathogens are inactivated, this feed can also be allowed for fish feed, and it is not necessary with restrictions on the use of products from category 3 material (except the species barrier).

In Norway, it is used either silage ($pH \leq 4$) or fresh (frozen) raw materials in the production of fishmeal/oil from aquaculture raw materials. Silage as conservation method will reduce the

amount of contamination in raw materials. Fishmeal from wild caught fish has always been produced from fresh raw materials with no use of silage.

The Norwegian Food Safety Authority (NFSA) has requested an assessment of how the pH, especially pH ≤ 4 and pH 7, will affect the temperature required for sufficient inactivation of fish disease agents.

4.2. Data

NFSA refer to the previous report, "Assessment of a method for treatment of category 2 and 3 materials of fish origin"(Norwegian Scientific Committee for Food Safety, 2010). Based on this report, the NFSA recommended a fish silage processing method (FSPM), silage, particle size ≤ 10 mm, with heating (≥ 85 ˚C for ≥ 25 minutes at pH ≤ 4), as hygienically safe to produce fishmeal and oil by-products from farmed fish, also from farms with disease restrictions (category 3).

In addition, the NFSA recommended that the processing of fresh raw materials, (no silage) can also be processed at 85 ˚C in 25 minutes. This assessment is based on the fact that IPN virus, which is considered to be the most resistant pathogen, is not inactivated at low pH but is inactivated at sufficiently high temperature and time.

This recommendation from the NFSA is applicable until satisfactory documentation of new relevant knowledge becomes available.

4.3. New documentation

On behalf of the industry, Rubin has in 2010 published a report (Rubin nr 199), on the inactivation of IPN virus at various temperature / time regimes in an appropriate matrix, and documented D and Z values for IPN virus. The report is a collaboration between Nofima and the National Veterinary Institute (NVI) and is available in the following link: http://www.rubin.no/index.php?current_page=nyheter&id=195

The NFSA consider this report as important basic knowledge for a scientific approach to establish a safe processing method for fishmeal and oil from both wild caught fish and aquaculture raw material.

Based on the documentation of inactivation of IPN virus in this Rubin report, the industry has asked the NFSA for approval of an updated method, "Standard Norwegian fishmeal and oil processing method", The method includes the use of raw material from wild-caught fish and from farmed fish. Due to the different risks of disease associated by the two types of raw materials, the industry will differentiate the temperature requirement for processing of the two raw materials.

Based on the conclusions in the report, the industry has requested to use 70°C for 20 minutes for processing of wild fish. It is concluded in the Rubin report that the use of 70°C for 20 minutes for processing of wild fish, will give a "100-$\log_{10}$" inactivation of Enterobacteriaceae and *Salmonella*.

For the processing of farmed fish, the industry has requested to use 76°C for 20 minutes or equivalent time / temperature combinations that results in a 3 $\log_{10}$ inactivation of the IPN virus.

Today there are discrepancies between the processing requirements recommended as safe by the NFSA for both silage and fresh by-products from farmed fish, particle size ≤ 10 mm, heating ≥ 85°C for ≥ 25 minutes, and particle size ≤ 10 mm, heating ≥ 76°C for ≥ 20 minutes, as the industry has requested to use for fresh (not silage) raw materials from aquaculture.

The processing requirements have to be proportionate to the risks associated with the possibility that raw materials can spread unwanted known and unknown fish pathogens.

Other relevant reports:

Inaktivering av *Salmonella* Rubin rapport nr 180
Inactivation of *Clostridium sporogenes* spores Nofima report 10/2011
Standard norsk fiskemel- og fiskeoljeprosess. Nofima rapport 35/2010 (norsk (33/2010
Engelsk versjon (33/2010)

4.4. Fish oil

The oil produced by a fishmeal-oil process may in practice be separated from the protein fraction before the mixture has reached a sufficient heat treatment. It may be desirable to have a more gentle treatment of fish oil to protect the quality of the oil. Adequate heating to obtain sufficient inactivation of fish pathogens in the oil fraction is important, also because there is no species restriction in the use of pure (protein free) fish oil.

The NFSA requests to know if the variation in matrix (meal / oil fraction) will affect the temperature required for sufficient inactivation of fish pathogens. Will the processing at a certain temperature give the same inactivation in a pure oil matrix as in a fishmeal mixture?

4.5. Microbiological regulatory requirements

Microbiological regulatory requirements for documentation of fishmeal do not include fish pathogens. There are only requirement related to *Salmonella* and Enterobacteriaceae, and for approval by method 7 in Regulation (EC) 1774/2002 (by-products regulation), also documentation of *Clostridium perfringens* is required.

Previously, there were national regulatory requirements (For 2007-03-29 No. 511) by 3 log 10 (99.9%) inactivation of *Aeromonas salmonicida*, *subsp. salmonicida* and IPN virus, in addition to the microbiological criteria applicable to fishmeal. This regulation is now repealed.

Available literature indicates IPN virus to be the most heat-and chemical-resistant fish pathogens, but is the inactivation of the IPN virus a suitable indicator for adequate inactivation of fish pathogens in general?

The NFSA would like an assessment of microbiological criteria suitable for validation of methods to secure a hygienic standard for the production of fishmeal and oil from the fish farming industry.

5. Terms of reference

NFSA has requested that the Norwegian Scientific Committee for Food Safety (VKM) conduct a risk assessment regarding the use of by-products from farmed fish in the feed for other farmed fish. NFSA has asked VKM to address the following questions. The final report should be written in English.

1. To which risks, in terms of listed diseases and infectious pancreatic necrosis (IPN) virus, will fish be exposed by use of untreated animal by-products (ABP), category 3?
2. Which risks to fish health will be presented by ABP category 3 materials following heat treatment at 76 °C for 20 minutes or 85 °C for 25 minutes?
3. What are the risks to fish health presented by fish oil or fishmeal originating from ABP category 3 materials after heat treatment at 76 °C for 20 minutes or 85 °C for 25 minutes?

4. What are the risks of infection presented by using processed salmon oil in salmon feed?
5. Is documentation of evidence of inactivation of the IPN virus a suitable indicator for sufficient inactivation of fish pathogens?
6. To what extent will silaging (pH equal to 4 or below) of by-products affect the answers given to the questions above?

In Norwegian:

1. Hvilken fiskehelserisiko representerer ubehandlede biprodukter kategori 3 fra norsk oppdrettsnæring? (Listeførte sykdommer og IPN).
2. Hva er fiskehelse risiko etter varmebehandling av biprodukter kategori 3 fra oppdrettsnæringen ved 76 grader i 20 minutter eller ved 85 grader i 25 minutter?
3. Hva er fiskehelse risiko i olje og/eller mel av biprodukter kategori 3 fra oppdrettsnæringen etter varmebehandling ved 76 grader i 20 minutter eller ved 85 grader i 25 minutter?
4. Hvilke smitterisiko innebærer bruk av bearbeidet lakseolje i fôr til laks?
5. Er dokumentasjon av inaktivering av IPN-virus en egnet indikator for tilstrekkelig inaktivering av fiskepatogener?
6. I hvilken grad vil ensilering (pH lik 4 eller mindre) av biproduktene påvirke svarene i spørsmålene overfor?

6. Assessment

6.1. Presumptions and general considerations

The risk assessment is based on the present fish health situation in Norwegian fish farming regarding infectious diseases.

Furthermore, the risk assessment only considers the use of animal by-products (ABP) of category 3 from Norwegian farmed fish, including fishmeal and fish oil made from such by-products, as ingredients in feed for farmed fish.

The use of the by-products of category 3 for any other purpose is not considered.

Any possible influence by or on wild fish or other wild fauna is not considered.

The assessment deals with the probability of the microorganisms in question being present and alive/active in the given material after various treatments. Whether the microorganisms present will infect fish and cause disease is not considered in this risk assessment, as this depends on both the state of the microorganisms and the state of the potential host animal.

It is presumed that the heat treated material has a core temperature of at least the specified temperature for at least the specified time. In the case of acidification of the material, it is presumed that the pH is 4.0 or less and obtained by the use of formic acid or another organic acid.

When assessing fish oil and fishmeal, it is presumed that the oil and meal has not been exposed to heat treatment with higher temperatures earlier in the production process.

The probabilities are categorized according to the scale:
negligible – very low – low – moderate – high – very high

In general, heat resistance of psychrotrophic bacterial and viral pathogens of fish has not been well examined. D- and z- values are determined for some of the fish pathogens, but many values are missing, making exact calculations needed to estimate inactivation at other temperatures impossible. Furthermore, the possible influence of the matrix in which the pathogenic organism is located when heat treated is often unknown. However, the Panel has anticipated effects of heat treatment on the different organisms based both on accessible knowledge on each specific organism, and general knowledge on heat treatment of microorganisms and influences of different matrix components.

Where D- and z-values are known, probable $\log_{10}$ reductions can be estimated for the required temperature and time. When the results of such calculations are reductions of very high $\log_{10}$ values, e.g. 30 $\log_{10}$, it is important to remember that these numbers are mathematical extrapolations far beyond what is scientifically acceptable. In the example, 30 $\log_{10}$ equals the estimated amount of bacteria in the whole world, and knowledge of heat treatment kinetics obtained in laboratory experiments cannot be extrapolated to fit such an enormous mass. However, such results can be used to indicate that the treatment is expected to be highly effective.

6.2. Hazard identification

This chapter contains an overview of the fish health situation in Norway and a list of microorganisms included in the assessment, followed by a description of the theoretical basis of heat treatment.

6.2.1. Fish health situation and the microorganisms included in this risk assessment

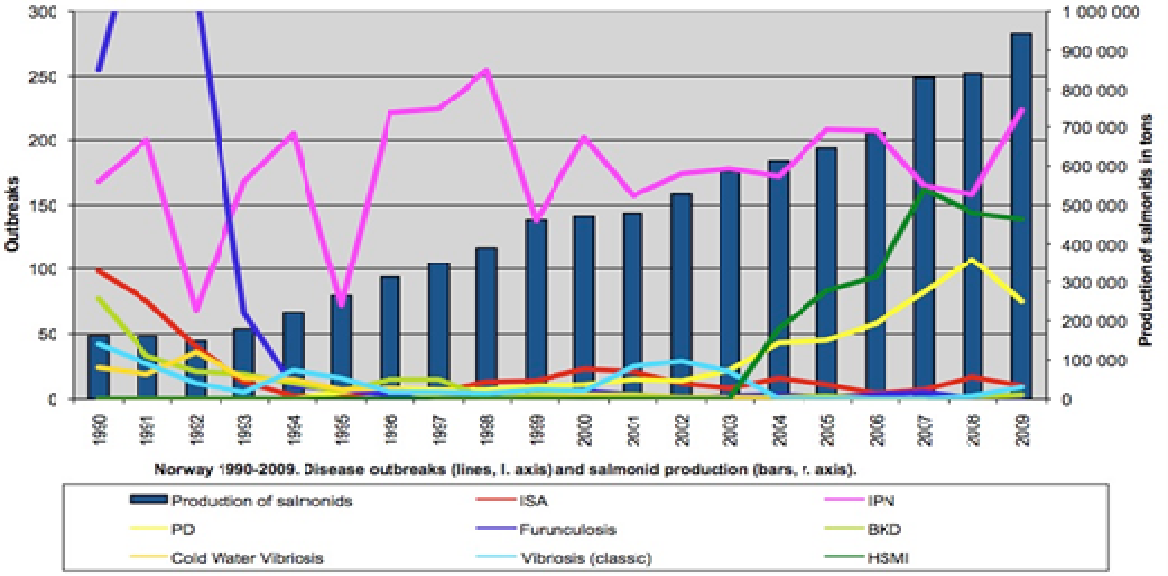

Figure 1. The number of recorded outbreaks of infectious diseases in salmon production in Norway 1990-2009. (Source: Norwegian Food Safety Authority).

The prevalence of disease outbreaks gives an indication of the prevalence and load of disease causing agents in the Norwegian aquaculture industry and thus hints for the potential risk of transmitting the infectious agent and/or disease by waste products. The virus diseases are the most prevalent. In number of outbreaks in the last decade the infectious pancreatic necrosis (IPN) has been the most dominating infectious disease, while both PD and HSMI can be categorized as emerging.

For regularly updated information on fish diseases in Norwegian fish farming, see the annual Fish Health Report from the Norwegian Veterinary Institute (http://www.vetinst.no/eng/Research/Publications/Fish-Health-Report)

In the terms of reference, the NFSA included "listed diseases and infectious pancreatic necrosis (IPN) virus", where "listed diseases" are defined in "Forskrift om omsetning av akvakulturdyr og produkter av akvakulturdyr, forebygging og bekjempelse av smittsomme sykdommer hos akvatiske dyr" (17.06.2008). During the risk assessment process, NFSA decided to exclude List 1 diseases in this risk assessment.

In addition, the Panel has included a few non-listed infections. The spore forming bacteria *Clostridum perfringens* and *Clostridum botulinum* were not included, as these do not cause List 2 or 3 diseases, and they have earlier been assessed in the (Norwegian Scientific Committee for Food Safety, 2010).

The organisms included in this risk assessment are listed in Table 1.

Table 1: Pathogens included in this risk assessment.

Type of organism	Organism	Disease	Classification*
Virus	Infectious hematopoietic necrosis virus (IHNV)	Infectious hematopoietic necrosis (IHN)	List 2
Virus	Viral hemorrhagic septicaemia virus (VHSV)	Viral hemorrhagic septicaemia (VHS)	List 2
Virus	Infectious salmon anaemia virus (ISAV)	Infectious salmon anaemia	List 2
Virus	Nodavirus	Viral nervous necrosis (VNN)/Viral encephalopathy and retinopathy (VER)	List 3
Virus	Salmon pancreas disease virus (SPDV) also called Salmonoid alpha virus (SAV)	Pancreas disease (PD)	List 3
Virus	Piscine reovirus (PRV)	Heart and Skeletal Muscle Inflammation	List 3
Virus	Infectious pancreatic necrosis virus (IPN virus)	Infectious pancreatic necrosis (IPN)	Not on list
Bacteria	*Renibacterium salmoninarum*	Bacterial kidney disease (BKD)	List 3
Bacteria	*Aeromonas salmonicida* subsp. *salmonicida*	Furunculosis	List 3
Bacteria	*Francisella* sp.	Francisellosis	List 3
Bacteria	*Vibrio (Listonella) anguillarum*	Vibriosis	Not on list
Bacteria	*Moritella viscose*	Winter ulcer	Not on list
Bacteria	*Lactococcus garviae*	*L. garvieae* infective endocarditis	Not on list
Bacteria	*Yesinia ruckeri*	Yesiniosis or enteric redmouth disease	Not on list
Bacteria	*Flavobacterium phsychrophilum*	Bacterial disease of cold water	Not on list
Parasites	*Gyrodactylus salaris*		List 3
Parasites	*Lepeophtheirus salmonis* (Salmon louse)		List 3

* As classified in "Forskrift om omsetning av akvakulturdyr og produkter av akvakulturdyr, forebygging og bekjempelse av smittsomme sykdommer hos akvatiske dyr" (17.06.2008).

6.2.2. Theoretical basis of heat treatment

6.2.2.1. Thermal inactivation of microorganisms

Thermal heat inactivation of microorganisms may be carried out in various ways depending on time/temperature relationships during treatment. Both dry and moist heat treatments may inactivate microorganisms, viruses and parasites, but heat treatment will also influence the

properties of the product. Mild heat treatments are sometimes requested in order to obtain specific or optimized properties for end product.

Thermal treatment may also be combined with chemical treatment in order to produce synergistic inactivation effects or modify the eventual end-products.

The first step prior to establishing thermal processes is identification or designation of the most heat-resistant or target microorganism/enzyme on which the process should be based. This requires the microbiological history of the products or organic material and conditions under which it is subsequently stored rendering it somewhat product specific.

Heat resistant microorganisms are one of the main threats for the stability and safety of heat treated feeds and by-products. Therefore, to know and understand the target microorganisms for each group of feeds is of the utmost importance in order to establish adequate preservation treatments to guarantee both their safety and stability. An appropriate heat treatment designed to achieve a specified lethality of microorganisms is influenced by many factors, some of which can be attributed to the inherent resistance of microorganisms, while others are due to environmental influences. It has been shown that factors such as pH or a_w can significantly reduce the *D*-values, therefore it is necessary to perform studies in the specific matrix of the product to ensure that it is adequate to control pathogenic microorganisms and to ensure its stability during its shelf life against spoilage agents.

6.2.2.2. Thermal resistance

Evaluation of thermal resistance of the target microorganism must be determined under the conditions that normally prevail in the product/organic material. In order to use thermal destruction data in process calculation, they must be characterized using an appropriate model. Further, since organic material cannot be heated to process temperatures instantaneously, data on the temperature dependence of microbial destruction rate is also needed to integrate the destruction effect through the temperature profile under processing conditions. The various procedures employed for experimental evaluation of thermal destruction kinetics of microorganisms are summarized in (Stumbo 1973) and (Pfulg 1988).

Published results on thermal destruction of microorganisms generally show that they follow a first-order reaction indicating a logarithmic order of death. In other words, the logarithm of the number of microorganisms surviving a given heat treatment at a particular temperature plotted against heating time (survivor curve) will give a straight line. The microbial destruction rate is generally defined in terms of a decimal reduction time (**D value**) which is the heating time in minutes at a given temperature required to result in one decimal reduction in the surviving microbial population. In other words, D value represents a heating time that results in 90% destruction of the existing microbial population. Graphically, this represents the time between which the survival curve passes through one logarithmic cycle

For calculation of D-value the first order kinetics approaches for inactivation rate usually used. The first order kinetics mean that all bacteria in a population are equally sensitive to heat and will die with a constant rate.

By plotting the logarithm of the various D-values against the corresponding temperature we get a graph where z-value can be calculated from the slope (Z=-1/a). **Z-value** (temperature coefficient) is defined as the number of degrees of temperature (°C) necessary to change the D-value with a logarithmic unit (Figure 2).

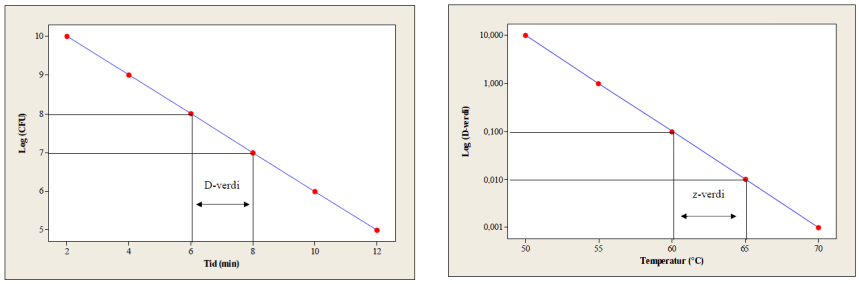

Figure 2. Basic plot of D - and z-values (Source: Jan Thomas Rosnes)

Heat process design is based on a suitable numbers of log reductions of the target organism which can be calculated using the D- and z-values.

6.2.2.3. Inactivation kinetics

The logarithmic nature of the survivor or destruction curve indicates that complete destruction of the microbial population is not a theoretical possibility, since a decimal fraction of the population should remain even after an infinite number of D values. In practice, calculated fractional survivors are treated by a probability approach; for example, a surviving population of 10^{-8}/unit would indicate one survivor in 10^{8} units subjected to the heat treatment.

Traditionally, in thermal processing applications, survivor counts of microorganisms are plotted directly on the logarithmic ordinate against time on the linear abscissa. The time interval between which the straight line portion of the curve passes through a logarithmic cycle is taken as the D value (Figure 2).

In the study by Nygaard and Myrmel (2010), IPN virus (serotype Sp) was heat treated in artificial media at pH 7 and 4. The inactivation curves are bi-phasic. The regression exponential curves (exposure time vs. Log_{10} IPN virus titre) from which D-values were calculated, were mainly determined by data from phase 2 of the biphasic inactivation. When considering the total inactivation of a certain temperature-time combination, the rapid initial 0.7 Log_{10} reduction had to be added.

6.2.2.4. Main factors important to heat resistance

The heat resistance of microorganisms is influenced by the medium in which it is exposed to heat. (van Asselt and Zwietering, 2006) collected 4066 published d-values for organisms involved in foodborne disease and linear regression was applied to obtain average D- and Z-values. When comparing these overall data it was seen that most factors reported to have an effect on D-value are smaller than the variability of all published D-values. A limited number of factors that did have a significant effect on D-value were identified. A high fat content protected bacteria against heat inactivation. It is also known that low water activity protects against thermal inactivation.

Experimental work has also shown that prior exposure to stresses such as alkaline or acid conditions may increase the heat resistance of bacteria (Humphrey, 1990; Humphrey et al., 1991; Farber and Pagotto, 1992; Humphrey et al., 1993; Casadei et al., 2001). Furthermore, the efficiency of treatments may be over-estimated in commercial conditions by ineffective sampling and testing (Williams, 1981) and the long tail of surviving low numbers of organisms which may be difficult to detect (Doyle and Mazzotta, 2000).

6.3. Hazard characterization

This chapter contains a hazard characterization and an assessment of heat treatment and acidification on each organism in Category 3 material, followed by a separate assessment of heat treatment of finished products, i.e. fishmeal and fish oil. A summary of hazard characterization is given in Table 3 at the end of the chapter.

6.3.1. Viruses

6.3.1.1. Infectious hematopoietic necrosis virus - IHNV

Characterization of the organism

IHNV belongs to the genus *Novirhabdovirus* of the family *Rhabdoviridae*. It has a linear, negative-sense, single-stranded RNA of approximately 11.1 kb. IHNV form two genogroups that are related to geographical location and not to year of isolation or host species (Kurath et al., 2003). Isolates from farmed rainbow (*Oncorhynchus mykiss* Walbaum) trout in Europe appear to have originated from North America (Enzmann et al., 2005).

Disease caused by the organism

Outbreaks of Infectious hematopoietic necrosis (IHN) often lead to very high mortalities characterized by haemorrhagic lesions in most organs. IHN affects wild and farmed rainbow trout, Pacific salmon including chinook (*O. tshawytscha*), sockeye (*O. nerka*), chum (*O. keta*), pink (*O. gorbuscha*), amago (*O. rhodurus*), masou (*O. masou*), and coho (*O. kisutch*), and Atlantic salmon (*Salmo salar L.*). Atlantic salmon is regarded as particularly susceptible. Other salmonids including species present in Norway like brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus L.*) have been reported to be somewhat susceptible by experimental infection.

As most other diseases there are variations in the spectra of clinical disease, and the virus may give persistent infections with no overt disease. The species and age of the fish are important determinants of the outcome of the infection.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

The virus has never been found in Norwegian farmed fish. If the virus gets established in Norwegian farming of salmonids, then untreated category 3 material from salmonids used as feed for salmonids may spread both virus and disease.

Current knowledge of the effect of heat inactivation and acidification

A complete inactivation is achieved at 55ºC for 30 sec (Whipple and Rohovec, 1994)

Complete inactivation at pH3.8-4.2 in in fish silage for 30 sec at 22ºC (Whipple and Rohovec 1994).

Anticipated effect of heating Category 3 material from Norwegian farmed fish to 76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

It can be anticipated that a complete inactivation of IHNV infectivity will occur in fish tissue homogenate kept at 76ºC for 20 min or 85ºC for 25 min.

The effect of pH at the temperatures of 76ºC or 85ºC most likely can not be tested due to rapid inactivation at those temperatures irrespective of pH.

6.3.1.2. Viral hemorrhagisk septikemi virus - VHSV

Characterization of the organism

VHSV belongs to the genus *Novirhabdovirus* of the family *Rhabdoviridae*. It has a linear, negative-sense, single-stranded RNA of approximately 11.1 kb. Genotyping based on G- and N-genes reveals four groups that often, but not always correlate with the geographical origin (Skall et al., 2005).

Disease caused by the organism

Outbreaks of VHS in farmed rainbow trout often lead to very high mortalities characterized by haemorrhagic lesions in most organs. There are variations in the spectra of disease, and the virus may give persistent infections with no overt disease. VHSV has been isolated from a vast number of both farmed and free-living marine and fresh water fish species. It is capable to cause mass mortality in free living fish species (Elsayed et al., 2006).

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

There is one reported outbreak in Norway for the last decades, in rainbow trout in 2007-2008 in Storfjorden, Møre og Romsdal. The virus belonged to Genotype III.

If the virus gets established in Norwegian farming of salmonids, then untreated category 3 material from salmonids used as feed for salmonids may spread both virus and disease. There is no such risk if today's situation with no VHSV in farmed fish withstands.

Current knowledge of the effect of heat inactivation and acidification

An inactivation of 99.9% is achieved at 50ºC for 10 min or 70ºC for 1 min (Vestergaard-Jørgensen, 1973).

An inactivation of 99.9% is achieved at pH2.5 for 10 min (Vestergaard-Jørgensen, 1973) or pH3 for 60 min (Ahne, 1982). Complete inactivation at pH3 for 180 min (Ahne, 1982).

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

It can be anticipated that a complete inactivation of VHSV infectivity will occur in fish tissue homogenate kept at 76ºC for 20 min or 85ºC for 25 min.

The effect of pH at the temperatures of 76ºC or 85ºC most likely can't be tested due to rapid inactivation at those temperatures irrespective of pH.

6.3.1.3. Infectious salmon aneamia virus – ISAV

Characterization of the organism

ISAV is a member of the *Orthomyxoviridae*, and is classified as the type species of the genus *Isavirus* within this virus family (Fauquet et al., 2005). It is an enveloped virus, and the genome consists of eight single-stranded RNA segments with negative polarity with a total size of approximately 14.3 kb. ISAV appears in two different virulence classes; one that causes disease and one non-virlent. The non-virulent viruses have a full-length variant of the surface protein hemagglutinin-esterase, and are called HE-HPR0 variants. Successful cultivation of ISAV with HE-HPR0 in cell cultures has not been achieved, and these variants have only been detected with PCR. Inactivation studies etc are therefore based upon results from the disease-causing, cultivable variants of ISAV.

Disease caused by the organism

ISA is a systemic disease affecting the circulatory system, and the major target cells for the infection are endothelial cells lining blood vessels. Final stages of the disease are characterised by hemorrhage, a circulatory collapse and an extreme anaemia. Experimental infections that are allowed to run may give an accumulated mortality of 100%.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

The number of ISA outbreaks in Norway culminated around 1990 with 80-90 annual outbreaks, this urged implementation of different important measures for controlling the disease. After that time the annual number of outbreaks has fluctuated between a few and 20, although a many-fold increase in salmon production has occurred simultaneously.

ISAV is present in Norwegian salmon farming industry. The virus may be present at a low load without overt disease being observed, particularly in rainbow trout (Snow et al., 2001). As a conclusion, ISAV may be present in category 3 material.

ISA is regarded as a species specific disease. In field conditions the disease has only been observed in Atlantic salmon. Other species, the most important being rainbow trout, may become infected naturally, and experimentally disease can be induced when the virus dose given has been large (Kibenge et al., 2006). In homozygote lines of rainbow trout high mortality after ISAV exposure has been achieved. It cannot be disregarded that rainbow trout may function as an intermittent reservoir for the virus after exposure.

If untreated category 3 material is used as feed for salmon it is possible to spread both virus and disease. If untreated Category 3 material is used as feed for rainbow trout it is possible to spread virus, but so far there are no examples of ISA disease in the field for this species.

Current knowledge of the effect of heat inactivation and acidification

ISAV does not replicate *in vitro* at temperatures of 25°C or above (Falk et al., 1997) and is rapidly inactivated at 37°C. In fish tissue homogenate infective virus could be detected after 55ºC 1 min, but not 55ºC for 2 min (Torgersen, 1999).

The virus in cell culture medium is stable in the pH range 5-9 (Falk et al., 1997). At pH 4, the virus is completely inactivated after 30 min.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and whether this will be affected by acidification to pH 4 or lower

It can be anticipated that a complete inactivation of ISAV infectivity will occur in fish tissue homogenate kept at 76ºC for 20 min or 85ºC for 25 min.

At optimum temperature (15ºC) pH 4, the virus is completely inactivated after 30 min. The effect of pH at the temperatures of 76ºC or 85ºC most likely can't be tested due to rapid inactivation at those temperatures irrespective of pH.

6.3.1.4. Nodavirus - Viral encephalopathy and retinopathy/Viral nervous necrosis (VER/VNN)

Characterization of the organism

Nodavirus are small, non-enveloped viruses, with a genome of linear, positive sense, two segments, single stranded RNA of a total of 4.5 kb. There are two genera *Alphanodavirus* and *Betanodavirus*. The viruses causing disease in fish belong to the *Betanodavirus* genus. Betanodaviruses affect a wide range of wild and farmed fish species globally, mostly from the marine environment.

Disease caused by the organism

Betanodaviruses causes infection mainly in young fish; affected organ is the central nervous system. Carriers of virus with covert infections are common. Virus can be transmitted vertically from one generation to the next.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

A limited number of disease outbreaks in Norwegian farmed marine fish are diagnosed annually. There does not seem to be any geographically restrictions. The virus may be present at a low load without overt disease being observed (Johansen et al., 2001). As a conclusion, Betanodavirus may be present in cateogory 3 material. Betanodavirus is not regarded to give disease in salmonids. However, there are reports of this (Grotmol et al., 1997), but these results have not been verified by other studies. If untreated category 3 material from a marine species is used as feed for a different marine species than the material originated from it is possible to spread both virus and disease. If untreated cateogory 3 material from marine species is used as feed for salmonids it is the common belief that this will not cause transmission of virus, but this is somewhat uncertain.

Current knowledge of the effect of heat inactivation and acidification

A complete inactivation is achieved at 70ºC for 10 min (Maltese and Bovo, 2001).

No inactivation at pH3 for 10 min (Arimoto et al., 1996). An inactivation rate of 90-99% was observed at pH2 for 15 days (Maltese and Bovo, 2001, Frerichs et al., 2000).

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

It can be anticipated that a complete inactivation of Betanodavirus infectivity will occur in fish tissue homogenate kept at 76ºC for 20 min or 85ºC for 25 min. Betanodavirus is quite resistant to acid conditions. No inactivation occurred at pH3 for 10 min (Arimoto et al., 1996). From this it is assumed that pH4 will have no effect on the heat treatment.

6.3.1.5. Salmon pancreas disease virus – SPDV (also called Salmonid alphavirus - SAV)

Characterization of the organism

SPDV belongs to the genus *Alphavirus*es in the family *Togaviridae* and is an enveloped virus with a linear, single-stranded positive sense RNA genome of approximately 12 kB. The virus was isolated for the first time in 1995 (Nelson et al., 1995), however the disease was known from the 1970-ies. SPDV is present in most European countries that farm salmonids (McLoughlin et al., 2007). The disease is called sleeping disease in freshwater rainbow farming. The term salmonid alphavirus (SAV) was introduced to cover both sleeping disease virus as well as pancreas disease virus. SAV and SPDV are therefore equal terms. SPDV is the name used by The International Committee on Taxonomy of Viruses, but SAV is most commonly used in literature in the last decade. Here, the term SPDV is used.

Sequence variations suggest that several genetically distinct populations of SPDV exist, and these are called subtypes 1-6 (Weston et al., 2005, Fringuelli et al., 2008). In Norway only subtype 3 (SAV3 or SPDV3) has been reported (Hodneland et al., 2005). {Alphaviruses of terrestrial animals are arthropod-borne (arbo-) viruses, which imply that alphaviruses, with the exception of SPDV, use arthropods as vectors in their transmission cycles.}

Disease caused by the organism

Infections with SPDV have been reported from farmed populations of Atlantic salmon and Rainbow trout both in freshwater and seawater (McLoughlin et al., 2007). Recently, SPDV was detected by PCR in several marine flatfish species such as common dab (*Limanda limanda*), long rough dab (*Hippoglossoides platessoides*) and plaice (*Platessa platessa*) (Snow et al., 2010). Moreover, genomic RNA has been detected in the parasitic arthropod Salmon lice (Lepeophtheirus salmonis) that were feeding on SPDV infected salmon, but no viral replication in this arthropod remains has been demonstrated (Petterson, 2009).

Disease caused by SPDV in Atlantic salmon is called Pancreas Disease, and in fresh water rainbow trout farms in central Europe the disease is called sleeping disease. The disease in rainbow trout in marine farms in Atlantic salmon raising areas is called pancreas disease.

The lesions caused by SPDV start out with pancreatic necroses are followed by inflammation and degeneration in cardiac and skeletal muscle. After experimental infections SPDV give intermittent viraemia (Christie et al., 2007) that disappears when neutralizing antibodies appear. During viraemia virus RNA is detectable by PCR in all tissues (Andersen et al., 2007).

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

In Norway, PD has only been reported from the marine phase of salmonid production (Jansen et al., 2010), despite reports of viral RNA in freshwater (Bratland et al., 2009). It is worthwhile to mention that SPDV in rainbow trout are frequently reported in freshwater fish stocks in Central Europe (McLoughlin et al., 2007). In Norway, most disease outbreaks, but not all, has occurred south of Hustadvika in Møre & Romsdal county. However, a limited number of outbreaks have occurred north of this border.

SPDV is present in Norwegian salmonid farming industry. The virus may be present at a low load without overt disease being observed (Hodneland and Endresen, 2006). As a conclusion, SPDV may be present in cateogory 3 material originating from both Atlantic salmon and rainbow trout. SPDV induced disease is not restricted to Atlantic salmon but also appearing in rainbow trout. There are no reports of this virus in other farmed fish species in Norway.

Consequently, if untreated category 3 material from salmonids is used as feed for salmonids it is possible to spread both virus and disease.

Current knowledge of the effect of heat inactivation and acidification

The virus is “rapidly inactivated” at 50ºC (McLoughlin et al., 2007). SPDV was reported to be “rapidly inactivated” in the presence of high levels of organic matter at 60 ºC at pH 7.2 and at pH 4 (Graham et al., 2007).

The virus is “rapidly inactivated” at pH 3.0 (McLoughlin et al., 2007).

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

It can be anticipated that a complete inactivation of SPDV infectivity will occur in fish tissue homogenate kept at 76ºC for 20 min or 85ºC for 25 min.

The effect of pH at the temperatures of 76ºC or 85ºC most likely can no be tested due to rapid inactivation at those temperatures irrespective of pH.

6.3.1.6. Piscine reovirus – PRV

Piscine reovirus – PRV is the assumed etiological agent of heart and skeletal muscle inflammation (HSMI) (Palacios et al., 2010). PRV is a reovirus, phylogenetically it falls between the genera of orthoreovirus and aquareovirus. Cultivation of the virus in cell cultures has not been successful and therefore there are no available information regarding inactivation. It is known that the virus causes a persistent infection of the fish (Løvoll et al., 2010). Based upon real-time PCR analysis the virus is very widespread in seawater reared farmed salmon, it has also been detected a few times in farmed salmon in the fresh water phase, and also in wild salmon (Palacios et al., 2010). It should therefore be assumed that PRV will be present in untreated Category 3 material from Norwegian farmed fish.

The orthoreoviruses and aquareoviruses are rather persistent viruses regarding survival outside the host. Therefore, from a general point of view, and also taking into consideration the widespread presence of PRV, indicate that PRV is persistent outside the host. However, there is no knowledge of the effect of heat inactivation and acidification of PRV.

6.3.1.7. Infectious pancreatic necrosis virus – IPN virus

Characterization of the organism

IPN is often referred to as the biggest health problem in Norwegian aquaculture.

The International Committee on Taxonomy of Viruses (ICTV) is doing the task of categorizing viruses. IPN virus is the type species of the genus *Aquabirnavirus* of the *Birnaviridae* family. According to the ICTV descriptions birnaviruses have a bi-segmented, (called segment A and B), double-stranded RNA genome total size of approximately 5.7 kb, which are contained within a medium-sized, non-enveloped, single-shelled, icosahedral capsid. The larger of the two genome segments, segment A, encodes a polyprotein which is cleaved to generate the major capsid polypeptides VP2 and VP3. The product of segment B is polypeptide VP1, the virion associated RNA-dependent RNA polymerase. VP is an abbreviation for the term “virus protein” and the number denotes the size, i.e. VP1 is the largest virus protein. The virus can be divided into virulent and less virulent variants based upon different amino acid motifs of the VP2.

It has previously been suggested that the term IPN virus should be used only for birnavirus isolated from salmonids and which causes IPN in these species, and that avirulent strains or virus isolates from different hosts with other disease conditions should be termed aquatic birnaviruses (Hill and Way, 1995; Reno, 1999; Skjelstad et al. 2003). Today, the name of a virus is based on genetic relationship and virus which clusters genetically with other IPN virus variants are called IPN virus.

Disease caused by the organism

IPN was primarily observed as an important cause of mortality in hatchery-reared salmonid fish fry (Wolf et al., 1971), found to occur in fish less than 5 g of size, characterized by necrosis of the pancreatic tissue. The clinical signs include dark body coloration, abnormal swimming behavior and swollen belly. However, in farmed Atlantic salmon, the disease is frequently observed in larger fish, particularly in connection with smoltification and transfer to seawater. Virus transmits efficiently horizontally, but also from one generation to next i.e. vertically. The mandatory disinfection process (commonly used is two treatments with 100 mg l^{-1} iodophore, for 10 min, in addition most (all?) eggs are routinely treated with formalin (0.01 %, 10-30 min) to prevent fungal infections, typically every second day - depending on the water quality) is not regarded as sufficient to completely stop this transmission.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

IPN virus is widely spread in Norwegian aquaculture. IPN disease has been diagnosed regularly and its occurrences somewhat fluctuating every year, as recorded by the Norwegian Veterinary Institute. As can be seen in Figure 1, it is considered the most dominating infectious disease in Norwegian aquaculture for the last 15 years. It causes disease in many species, and may be present in all farmed fish species in Norway. In 2009 there were 53 disease outbreaks in salmonid production in the fresh water phase and 170 in the marine phase. IPN is no longer a notifiable disease in Norway, so the number of outbreaks can be regarded as minimum numbers.

IPN virus causes disease in Atlantic salmon, rainbow trout, cod etc and may be present in category 3 material from any farmed fish species in Norway. If untreated category 3 material is used as feed for fish it is possible to spread both virus and disease. This is independent upon the requirement that such material should not be given to the same species as the category 3 material originated from.

When IPN virus was intraperitoneally injected in brook trout (*Salvelinus fontinalis*) an asymptomatic, persistent infection was induced in 100% of the fish. The fish stayed persistently infected for at least 76 weeks in spite of the production of a strong humoral immune response (Bootland et al., 1991). The virus is found intracellularly in persistently infected fish (Smail et al., 2003), and kidney is considered to be the organ containing most virus, but other organs contain virus as well (Yamamoto, 1975). The IPN virus titres in kidneys of healthy, persistently infected fish has been estimated to 10^2 to 10^4 $TCID_{50}$ g–1 tissue in Atlantic salmon when tested 6-8 weeks after infection (Johansen and Sommer, 2001) and approximately in the same range in brook trout that was tested 76 weeks post infection (Bootland et al., 1991). Experiments in Atlantic salmon indicate that the infective dose may be as low as 1 CTID50 by bath immersion (Urquhart et al., 2008).

Current knowledge of the effect of heat inactivation and acidifaction

The infectivity of IPN virus is preserved outside the fish host for a long period of time, and also at a relatively large pH range. Experiments using IPN virus suspended in buffers reported a 3 log_{10} reduction at 4ºC after 1 yr. After 2 years, viable viruses were still present (Wolf and Quimby, 1971). The reported survival of IPN virus after passage through mammals/birds (Smail et al., 1993) is related to its ability to withstand prolonged exposure to low pH and to its relative heat stability. Thermal inactivation of IPN virus has been described by several authors to occur in

two steps, first a rapid inactivation and then a slower inactivation of the rest of the virus. A summary of the literature of thermal inactivation of IPN virus is given in the report: "Inactivation of pathogenic microorganisms in fish by-products Sub-project IPN virus" (Nofima, NVI, 2010 *ISBN:* 978-82-7251-801-0 (pdf)).

In the Nofima/NVI report and a later paper based on the report (Nygaard et al., 2010), thermal inactivation of IPN virus as well as the effect of pH 4 and 7 on the inactivation was tested. A short discussion on the methods used to test the presence of IPN virus in that report is given here: They rejected the use of real-time PCR or classical PCR as a method for detection of viable IPN virus, and preferred the use of cell culture for detection of this. The reason for this is that the dsRNA of IPN virus was still detectable by PCR methods even after 24 h at 85 ºC, although a 4-5 Log reduction in number of RNA copies was observed. On the other side, an estimated reduction in viable IPN virus at 85 ºC was 27 log_{10} after 25 min. Therefore, the authors concluded that detection of IPN virus by PCR did not reflect detection of viable virus.

This could, of course, have been somewhat helped by using longer targets in the PCR method, but in general we find that report's conclusion in this matter to be sound.

Furthermore, to avoid the toxic effect that homogenized fish tissue may have on fish cell cultures (could for instance be immunological active substances in fish tissue that are executing this) they made an artificial matrix in which the virus was suspended. This raises a further question if the artificial matrix they used resembles Category 3 material. To get a matrix with protein content approximately similar to fish tissue homogenate they used bacteriological peptone as protein source (protease digested animal proteins). There is a substantial amount of fat in fish tissue, which was not present in their artificial medium; however, the authors estimated the concentration of IPN virus in the fat fraction of virus containing fish tissue homogenate to be 1 $\log_{10}$ less than that of the water soluble fraction. In fish tissue homogenate it will be natural substances that will reduce the infectivity of IPN virus; this may for instance be circulating neutralizing antibodies or other components of the immune system. Similar factors, specifically reacting with IPN virus, cannot be anticipated to be present in the peptone solution. The results from the Nofima/NVI report indicated that the fish suspension reduces the IPN virus infectivity. In conclusion; although they used an artificial peptone solution to omit negative effects of fish tissue homogenate on their testing system we cannot find that this would have big impact of the validity of their results.

At pH 4 they found that a 3 $\log_{10}$ reduction was achieved by 60ºC after 13 h, 75ºC for 23 min, 80ºC for 7.1 min, 85ºC for 2.2 min. At 75ºC or 85ºC, pH 4, the time to obtain 10-fold reduction (D-value) of IPN virus infectivity was 10 min and 0.9 min, respectively.

Current knowledge of the effect of acidification

IPN virus has normally been regarded as stable between pH 3-9. Exposure to pH 3.8, at 4°C only gave a reduction in virus titre from 8.3 to 4.2 $\log_{10}$ pfu/ml and from 6.48 to 3.87 $\log_{10}$ pfu/ml with two different isolates over a 147 day period, and several hours were required to produce a 3 $\log_{10}$ reduction in infectivity at pH 2.5 (Smail et al., 1993). In the above mentioned Nofima/NVI report a slightly better maintenance of the infectivity of IPN virus was found at pH 4 than at pH 7. They found heat resistance of IPN virus was only little influenced by pH 4 or pH 7, i.e. the exposure temperatures must be 1,6 ºC higher at pH 4,0 than at pH 7,0 in order to achieve the same inactivation.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Estimated reduction in IPN virus infectivity at 76ºC for 20 min in fish tissue homogenate at pH 4 or pH 7 is 3 $\log_{10}$. Estimated reduction in IPN virus infectivity at 85ºC for 25 min in fish tissue homogenate at pH 4 or pH 7 is 25-27 $\log_{10}$.

The lowering of pH from 7 to pH 4 will not induce more rapid heat inactivation of IPN virus. If any influence at all, the infectivity of IPN virus is slightly improved at pH 4 compared to pH 7. There is a lack of data to firmly state the effect of pH lower than 4 for inactivation of IPN virus.

6.3.2. Bacteria

An overview of published and reported data on thermal inactivation and survival at pH 4 of the bacteria covered in this section is displayed in Table 2 and 3.

6.3.2.1. *Renibacterium salmoninarum*

Characterization of the organism

Renibacterium salmoninarum is an aerobic, non-motile, non-spore forming, Gram-positive short rod or diplobacillus. The organism is slow growing and optimal growth occurs at 15 - 18°C (Sanders and Fryer, 1986).

Disease caused by the organism

The bacterium is the causative agent of Bacterial Kidney Disease (BKD), a chronic condition reported in salmonids (Fryer and Sanders, 1981).

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

The disease is widely distributed throughout North and South America, Europe and Japan. Disease transmission is thought to occur both horizontally via infected water or infected food and vertically from parent to progeny via infected ova. According to Fiskehelserapporten (2010) BKD was not established in Norway in 2010.

Current knowledge of the effect of heat inactivation and acidification

Whipple and Rohovec (1994) demonstrated that a suspension of 10^7 or 10^8 colony forming units (cfu) ml^{-1} of *R. salmoninarum* in phosphate buffered saline (PBS), titers of the bacterium decreased after a few min at 50 and 55°C but survived in low numbers for more than 4 hours at 50°C, for 3 hours at 55°C and for 15 min at 65°C. The authors stated that titers decreased after a few minutes at these temperatures but that low numbers of bacteria survived for the periods stated. However precise data on titers reduction or D-values were not supplied. The authors also reported that from the initial titers, *R. salmoninarum* could not be detected in fish silage (pH 4.0) after 30 min (the first sampling time) at 55°C. Smail et al. (1993) assessed survival of *R. salmoninarum* (>10^8 cfu ml^{-1}) in fish silage, a mixture of formic and propionic acid; however no proportions of mixture was given. *R. salmoninarum* was not isolated from plates or broths after mixing in silage even though several samplings were taken at intervals of; 0, 15, 30, 90, 180 and 240 min. As the experiment was not carried out in an acceptable manner it is not possible to present a clear conclusion with respect to D-values and precise inactivation rate.

To our knowledge, only one study has evaluated the survival of *R. salmoninarum* at pH 4 (Defra, 2005). The results from this study are shown in Table 3.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Complete inactivation is anticipated in culture medium at 76ºC for 20 min. A possible protective effect of high fat content is not known, but due to the very low prevalence of *Renibacterium salmoninarum* in Norway, the Panel anticipates the probability of this bacterium being present following heat treatment of ABP category 3 material both at both 76°C for 20 minutes and at 85ºC for 25 min to be negligible. Acidification to pH 4 or lower will contribute to increased bactericidal effect.

6.3.2.2. *Aeromonas salmonicida* subsp. *salmonicida*

Characterization of the organism

A. salmonicida subsp. *salmonicida* is a Gram – negative facultative anaerobic, non-motile, non-spore forming rod. Optimum temperature of the bacterium is between 22 – 28°C but some strains do not grow at 35°C (Popoff, 1984).

Disease caused by the organism

The organism is the causative agent of "*typical*" furunculosis in salmonid fish and a range of ulcerative and other conditions in non-salmonid fish species. The organism, which was first isolated in the 19th century, has a virtually worldwide distribution and is a major source of losses in wild and farmed fish (Birkbeck and Ringø, 2005; Bøgwald and Dalmo, 2012), but the bacterium has also been reported to be a part of the intestinal microbiota of fish (Ringø et al., 1997; Kim et al., 2007). Infection is transmitted horizontally via infected water or ingestion of infected food and the organism can survive in water and sediments for periods of weeks or months. Relatively effective vaccines are now available for salmonid fish.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

Disease outbreak caused by *A. salmonicida* subsp. *salmonicida* or atypical *A. salmonicida* was not reported in salmonids in Norway in 2010 (Fiskehelserapporten, 2010), and the anticipated presence in category 3 material is anticipated to be very low.

Current knowledge of the effect of heat inactivation and acidification

Smail et al. (1993) reported that *A. salmonicida* was rapidly inactivated in commercial fish silage containing a mixture of formic - and propionic acid. The authors reported that from an initial viable count of 5x10^{8} cfu ml^{-1} the bacterium could not be re-isolated from plates or broth after mixing in the silage. No D-values were provided. Whipple and Rohovec (1994) reported that from an initial viable count of 1.4 x 10^{8} cfu ml^{-1} *A. salmonicida* could not be re-isolated from fish silage (pH 4.0) after 3 min at 22°C, but the bacterium survived for 90 min in the low pH buffer (citric phosphate). The authors also reported that a suspension of approximately 10^{8} cfu bacteria ml^{-1} in PBS was completely inactivated after 2 min at 50°C, although no D-values were provided.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to 76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Complete elimination following heat treatment of ABP category 3 material both at both 76°C for 20 minutes and at 85ºC for 25 min is anticipated. Acidification to pH 4 or lower will contribute to increased bactericidal effect.

6.3.2.3. *Francisella* sp.

Characterization of the organism

Bacteria from the genus *Francisella* are characterized as strictly aerobic, facultatively intracellular, non-motile, Gram-negative small coccobacilli or rod-shaped (Foley and Nieto, 2010). They are typically catalase positive and oxidase negative and optimum temperature is 37°C (Eigelsbach and McGann, 1986). The genus *Francisella* is part of the family Francisellaceae, order Thiotrichales, of the sub-class γ-Proteobacteria. Tularemia, also known as rabbit fever, is caused by the bacterial pathogen *Francisella tularensis* and the disease was defined by McCoy (1911).

Disease caused by the organism

Francisella sp. is an emergent bacterial pathogen that causes acute to chronic disease in warm and cold water cultured and wild fish species and in shellfish (Birkbeck et al., 2011). Infected fish present non-specific clinical signs such as erratic swimming, anorexia, anemia, exophthalmia and high mortality. Upon macroscopic and microscopic examination, several internal organs are usually enlarged and contain widespread white nodules in the affected tissue. Histological examination of this tissue reveals the presence of multifocal granulomatous lesions, with numerous small, pleomorphic, cocco-bacilli.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

In Norway, Atlantic cod is considered the susceptible species for *F. noatunensis* subsp. *noatunensis.* The presence in category 3 material from farmed Atlantic cod is anticipated to be moderate to high.

Current knowledge of the effect of heat inactivation and acidification

Thermal resistance of *Francisella tularensis* has been studied in infant formula and fruit juices. In infant formula D-values ranged from 12 s at 57.5°C to 580 s at 50°C with a z-value of 4.37°C for the strain tested. Furthermore, the strain tested did not survive at temperatures above 55°C in mango juice and orange juice (>6-log inactivation) (Day et al., 2008). To our knowledge, no data on thermal inactivation or inactivation with acid has been reported regarding *Francisella noatunensis* subsp. *noatunensis*.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Based on information obtained on related species, complete elimination following heat treatment of ABP category 3 material both at both 76°C for 20 minutes and at 85ºC for 25 min is anticipated.

6.3.2.4. *Vibrio*

Characterization of the organism

Members of the genus *Vibrio* are Gram-negative, oxidase-positive, non-spore forming and are typically slightly curved rods. Genus *Vibrio* is indigenous and widespread in aquatic habitats at different salinities and they are common in marine and estuarine environments, and on the surfaces of marine plants and animals (Baumann et al., 1984; West, 1989; Drake et al., 2007; Austin, 2010). All vibrios grow at 20^{o}C and most at 30^{o}C (Baumann et al., 1984). Research carried out in the 70ties and 80ties suggested that genera of *Vibrio* belonged to the dominant culturable heterotrophic gut microbiota especially in marine fish (Sakata, 1990). However, more recent studies based on the use of molecular methods have questioned this statement (for review see, Ringø et al., 2012).

There are 30 species in the genus *Vibrio* and 13 of these are pathogenic to humans (Austin, 2010). Important fish and shellfish pathogenic vibrios are *Vibrio anguillarum* (currently *Listonella anguillarum*), *Vibtio ordalii, Vibrio salmonicida* (currently *Allivibrio salmonicida*), *Vibrio splendidus*, *Vibrio viscosus* (currently *Moritella viscosa*) and *Vibrio vulnificus* (Lillehaug et al., 2003; Birkbeck and Ringø, 2005; Bøgwald and Dalmo, 2012). To our knowledge there is also information available displaying that *V. anguillarum* and *V. salmonicida* are detected/isolated in fish intestine (Grisez et al., 1996; Zhou personal communication, 2011).

6.3.2.4.1. *Vibrio (Listonella) anguillarum*

Disease caused by the organism

V. anguillarum, tend to cause disease at higher water temperatures while *V. salmonicida* only cause disease at low water temperatures (Woo and Bruno, 1999).

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

The presence in category 3 material is anticipated to be moderate.

Current knowledge of the effect of heat inactivation and acidification

A 4 $\log_{10}$ reduction of *V. anguillarum* has been reported following exposure of the organism to a temperature of 44°C for 3 min and for 2 min at 47.5°C in seawater (Jacobsen and Liltved, 1988). Jacobsen et al. (1989) reported that a suspension of 10^6 *V. anguillarum* ml^{-1} in filtered fish slaughterhouse wastewater was inactivated after 1 min at 60°C and after 15 seconds at 72°C.

To the Panel's knowledge, no data is available on inactivation with acid regarding *V. anguillarum*.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Complete elimination following heat treatment of ABP category 3 material both at both 76°C for 20 minutes and at 85 ºC for 25 min is anticipated.

6.3.2.4.2. *Moritella viscosa*

Disease caused by the organism

M. viscosa is considered the main aetiological agent of *winter ulcer*, a disease that affects farmed salmonid fish at temperatures below 7-8°C (Lunder, 1992; Lunder et al., 1995). According to Bjornsdottir et al. (2011) knowledge of its pathogenicity is limited and there are no reports comparing the virulence properties of a collection of bacterial isolates.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

The presence in category 3 material is anticipated to be high.

Current knowledge of the effect of heat inactivation and acidification

To our knowledge, no data on; thermal inactivation or inactivation with acid has been reported regarding this pathogen.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Bases on information obtained on related species, complete elimination following heat treatment of ABP category 3 material both at both 76°C for 20 minutes and at 85ºC for 25 min is anticipated.

6.3.2.5. *Lactococcus garvieae*

Lactococci are Gram-positive, microaerophilic cocci which grow well at 37°C (Hardie, 1986). They can simply be differentiated from pediococci and leuconostocs by the main fermentation products from glucose. *Lactococcus garvieae* is an important pathogenic bacterium of worldwide significance against which commercial vaccines are available (Wang et al., 2007; Gatesoupe, 2008).

Disease caused by the organism

An early study suggested that *L. garvieae* was considered as the most important risk factor for the Mediterranean trout industry, causing losses of was approximately 50 % of the total production (Ghittino and Praero, 1992). Warm water infections (>15°C) caused by *L. garvieae* occur in both cultured freshwater and marine fish (Lauzon and Ringø, 2011). The bacterium has also sporadically been reported to be a part of the intestinal microbiota of common carp (*Cyprinus carpio*) and freshwater prawns (*Macrobrachium rosenbergii*) (Cai et al., 1999). Disease outbreaks, which usually manifest as a haemorrhagic septicaemia, are most common in the summer months when water temperatures are highest.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

L. garvieae infection has not been reported in Norwegian aquaculture.

Current knowledge of the effect of heat inactivation and acidification

To our knowledge, only one report is available on thermal inactivation and inactivation with acid (pH 4) regarding this pathogen (Defra, 2005). The results from this study are shown in Table 2 and 3. The inactivation studies in the Defra report were conducted at two separate laboratories, CEFAS Laboratory Dorset and Fisheries Research Service Laboratory, Aberdeen, and the results showed that *L. garvieae* was the most resistant bacterium tested to 60°C and different pH (4 and 12). However, as cultivation evaluations using Tryptic Soy Agar and Broth were used in this study we recommend that evaluations using molecular techniques merits further investigations.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Complete inactivation is anticipated in culture medium at 76ºC for 20 min. A possible protective effect of high fat content is not known, but due to *L. garvieae* not being present in Norway, the Panel anticipates the probability of this bacterium being present following heat treatment of ABP category 3 materials at 76°C for 20 minutes and at 85ºC for 25 min to be negligible. Acidification to pH 4 or lower will probably not contribute to increased bactericidal effect.

6.3.2.6. *Yersinia ruckeri*

Characterization of the organism

Yersinia ruckeri belongs to the Enterobacteriaceae family and is a Gram-negative rod of variable motility and has an optimum growth temperature range of 22 – 25°C, although the organism grow between 9 - 37°C (Bercovier and Mollaret, 1984).

Disease caused by the organism

Y. ruckeri is the causative agent of yersiniosis or enteric redmouth disease (Furones et al., 1993).

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

In 2010, the disease was reported in 12 salmon locations in Norway (Fiskehelserapporten, 2010). The presence in category 3 material is anticipated to be low.

Current knowledge of the effect of heat inactivation and acidification

Some information is available on thermal inactivation of *Y. ruckeri* (Jacobsen et al., 1989; Smail et al., 1993; Defra, 2005). Jacobsen et al. (1989) reported that a suspension of $6.6x10^7$ *Y. ruckeri* per ml in filtered slaughterhouse waste water was inactivated after 1 min at 60°C and after 15 seconds at 72°C while the Defra report recorded approximately 5 $\log_{10}$ inactivation of *Y. ruckeri* after 1 hour at 60°C. In the study of Smail et al. (1993) the authors showed that using silage less than 10 colonies of the bacterium were detected on the zero time plates.

To our knowledge, only one study has evaluated the survival of *Y. ruckeri* at pH 4 (Defra, 2005). The results of this study are displayed in Table 3.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

Complete elimination following heat treatment of ABP category 3 material both at both 76°C for 20 minutes and at 85 ºC for 25 min is anticipated. Acidification to pH 4 or lower will contribute to increased bactericidal effect.

6.3.2.7. *Flavobacterium psychrophilum*

Characterization of the organism

Bacteria belonging to the *Flavobacterium* genus are; Gram-negative, aerobic and grow at temperatures from 5 - 30°C; most clinical isolates also grow at 37°C (Holmes et al., 1984).

Disease caused by the organism

F. psychrophilum causes "bacterial disease of cold water" on juvenile rainbow trout (Leon et al., 2009). The authors reported that ulceration of the dorsal area was the most frequent macroscopic external injury as well the localized blackening of the skin, while internally there was a marked splenomegaly, pallor of the liver, kidney and gills and inflammation of the intestine.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

According to the data reported in Fiskehelserapporten (2010) few disease outbreaks caused by *F. psychrophilum* were reported in Norway in 2010. The bacteria infect mainly rainbow trout and occasionally salmon. The presence in category 3 material from rainbow trout is anticipated to be low to moderate.

Current knowledge of the effect of heat inactivation and acidification

To our knowledge, no report is available on thermal inactivation and inactivation with acid regarding this pathogen.

Table 2: Thermal inactivation of bacterial fish pathogens.

Bacterial species	Initial viable counts	Matrix	Temp (°C)	Effect	References
R. salmoninarum	$>10^8$ ml^{-1}	Silage	NI	Not detected after mixing in the silage	Smail et al. (1993)
R. salmoninarum	$7.5x10^8$ ml^{-1}	Culture medium	60	>5.7 $\log_{10}$ drop in 5 min	Defra (2005)
A. salmonicida	$5x10^8$ ml^{-1}	Silage	NI	Not detected after mixing in the silage	Smail et al. (1993)
A. salmonicida	$1.4x10^8$ ml^{-1}	Silage	22	Not detected after 3 min	Whipple and Rohovec (1994)
A. hydrophila	~10^8 ml^{-1}	Culture medium	60	D-value: 0.026 to 0.04 min	Schumann et al. (1997)
V. anguillarum	10^6 ml^{-1}	Filtered fish slaughterhouse waste water	60	Inactivated after 1 min	Jacobsen et al. (1989)
V. anguillarum	10^6 ml^{-1}	Filtered fish slaughterhouse waste water	72	Inactivated after 15 sec	Jacobsen et al. (1989)
V. vulnificus	10^6 ml^{-1}	Oyster homo-genates	46	D-value: 1.3 min	Dombroski et al. (1999)
V. vulnificus	10^6 ml^{-1}	Oyster homo-genates	48	D-value: 0.41 min	Dombroski et al. (1999)
V. vulnificus	10^6 ml^{-1}	Oyster homo-genates	50	D-value could not be detected due to quickly inactivation.	Dombroski et al. (1999)
L. garvieae	$1.3x10^8$ ml^{-1}	Culture medium	60	D-value: 3.31	Defra (2005)
Yersinia ruckeri	$6.6x10^7$ ml^{-1}	Filtered fish slaughterhouse waste water	60	Inactivated after 1 min	Jacobsen et al. (1989)
Y. ruckeri	$6.6x10^7$ ml^{-1}	Filtered fish slaughterhouse waste water	72	Inactivated after 15 sec	Jacobsen et al. (1989)
Y. ruckeri	$2x10^9$ ml^{-1}	Silage	NI	< 10 colonies were detected on plates sampled immediately after mixing in silage	Smail et al. (1993)
Y. ruckeri	$<2.5x10^7$ ml^{-1}	Culture medium	60	D-value: >4.95	Defra (2005)

(NI – no information was given)

Table 3: Survival of three bacterial fish pathogens exposed to pH 4 for 24 h (after Defra 2005).

Bacterial species	Initial viable counts	Matrix	Temp (°C)	Drop in concentration (log_{10})
R. salmoninarum	$7.9x10^7$ ml^{-1}	Culture medium	NI	4.75
L. garvieae	$1.7x10^9$ ml^{-1}	Culture medium	NI	0.25
Y. ruckeri	$7.7x10^8$ ml^{-1}	Culture medium	NI	3.95

NI – no information was given.

6.3.3. Parasites

6.3.3.1. *Gyrodactylus salaris*

Characterization of the organism

Gyrodactylus salaris belongs to the Monogenea, a group of small parasitic flatworms mainly found on skin and gills of fish. Monogeneans attach to the host using hooks, clamp and a variety of other specialised structures. *G. salaris* is a viviparous freshwater parasite that mostly lives on the skin and fins of Atlantic salmon pre-smolt stages. In Norway, *G. salaris* has also been found in farmed rainbow trout, wild Arctic charr and wild brown trout.

Disease caused by the organism

Clinical signs. Usually there are no clinical signs in fish with one or up to a few tens of parasite specimens. In the early disease phase, increased flashing (fish scratch their skin on the substrate) is typical. Later, fish may become greyish because of increased mucus production and the fins may be eroded. Diseased fish are lethargic and are usually found in slower-moving water.

Behavioural changes. Flashing is common among moderate to heavily infected farmed fish as they scratch their skin in the bottom or wall of a tank or pond. Heavily infected fish may have reduced activity and stay in low current areas.

Gross pathology. Heavily infected fish may become greyish as a result of increased mucification, and at a later stage the dorsal and pectoral fins may become whitish as a result of increased thickness (mainly hypertrophy) of the epidermis. Heavily infected fish may have eroded fins, especially dorsal, tail and pectoral fins, because of parasite feeding. Secondary fungal infections (*Saprolegnia* spp.) are commonly observed in fish with gyrodactylosis.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

Gyrodactylus salaris was introduced to Norway in the early 1970s and was observed for the first time in 1975. During the period of 1975 to 2011 *Gyrodactylus salaris* has been detected on Atlantic salmon fingerlings/parr from 48 rivers, 13 hatcheries/farms with Atlantic salmon parr/smolts and 26 hatcheries/farms with rainbow trout. In addition, a *G. salaris* strain has been found on Arctic charr in three lakes in Nordland County and in several lakes in southern Norway.

The policy of the Norwegian Authorities is to eradicate *G. salaris* from infected rivers and farms. The procedure is to eliminate all the hosts (salmon and rainbow trout). By doing so, the parasite is also eliminated as it has no specialized free-living stages or use intermediate hosts. In rivers, rotenone has been used to kill all fish hosts while stamping out of all fish have been

used in farms. By September 2011, *G. salaris* was confirmed to be eradicated from 21 rivers and from all hatcheries/fish farms. Three rotenone treated rivers are under surveillance for freedom of the parasite. The parasite is known or suspected to be present in 24 rivers in Norway. The southernmost infected river is the river Sandeelva in Buskerud county and the northernmost is the river Skibotnelva in Troms county.

The probability that *G. salaris* being present in category 3 material is negligible.

Current knowledge of the effect of heat inactivation and acidifaction

Deactivation of *G. salaris* by heating has not been studied. However, in a small test the parasites were immediately killed in hot tap water (approximately 50°C) (unpublished result).

Deactivation of *G. salaris* in low pH solutions has not been studied. However, in a laboratory studies all *G. salaris* disappeared from salmon parr in pH 5 while the fish were still alive (Soleng et al., 1999).

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

The survival of *G. salaris* in silage has not been studied. However, the Panel believes that G. salaris will not survive in Category 3 material heated to 76°C and higher, and that acidification to pH 4 or lower will not have any additional effect.

6.3.3.2. *Lepeophtheirus salmonis* (Salmon louse)

Characterization of the organism

Lepeophtheirus salmonis belongs to the Copepoda, a species rich group of free-living and parasitic crustaceans. Parasitic copepods are mainly found on skin and gills of fish using specialised and modified structures for attachment to the host. *L. salmonis* is an egg-laying saltwater parasite living on the skin and fins of salmonids in their marine growth phase.

Disease caused by the organism

L. salmonis feeds on skin, mucus and blood. The feeding activity results in skin erosion. The severity of parasite feeding is dependent on parasite numbers and stage and fish size. In severe cases fish dies mainly due to osmotic imbalance. In general, fish with less than 10 lice will not be considered diseased. Due to national regulations, farmed salmonids have less than 4-5 salmon lice (all stages). Thus, disease outbreaks in farms or even individual fish due to *L. salmonis* are rare.

Anticipated presence of the organism in untreated Category 3 material from Norwegian farmed fish

L. salmonis is present on salmonids along the whole Norwegian coast. The parasite infects wild Atlantic salmon, sea trout and sea run Arctic charr, and farmed and escaped Atlantic salmon and rainbow trout.

It is not probable that *L. salmonis* can survive in untreated category 3 material used in feed.

Current knowledge of the effect of heat inactivation and acidification

The effect of heating to *L. salmonis* has not been studied in detail but based on general knowledge, it can be stated that these parasites are heat sensitive. Most certainly, salmon lice die at temperatures above 50°C or even lower. Parasite eggs are often more resistant to chemical and physical changes. However, salmon louse egg are thin shelled and metabolically active, and little tolerant to changes. For example, the eggs are O_2 sensitive and quickly die

when O_2 concentration in the water is reduced. This is observed when fish are killed by CO_2 prior to slaughtering. Most lice and lice eggs die in this process (Heuch, pers. comm.).

Deactivation of *L. salmonis* in low pH solutions has not been studied.

Anticipated effect of heating Category 3 material from Norwegian farmed fish to76ºC for 20 min or 85ºC for 25 min, and the possible influence on this effect by acidification to pH 4 or lower

The survival of *L. salmonis* in silage has not been studied. However, the Panel believes that *L. salmonis* (any stage including the egg) will not survive in Category 3 material heated to 76°C and higher, and that acidification to pH 4 or lower will not have any additional effect.

6.3.4. Heat treatment of finished products

6.3.4.1. Fishmeal

Microorganisms can survive but not multiply in fishmeal. However, the microbiological stability of fishmeal solely depends on its low moisture content. During handling and storage, finished fishmeal must be carefully protected against moisture from water leakage or water vapour condensation.

The water content of fishmeal is typical 6% and maximum 10%. 10% water in fishmeal corresponds to an Aw (water activity) of approximately 0.60. Most bacteria require Aw above 0.95 corresponding to 30 – 35 % water in order to grow. According to experience, fishmeal containing up to 20% water appears to be microbiological stable but should still contain maximum 10% for other reasons (flow properties, oxidation, etc.). Low water activity is known to offer bacteria a better protection against heat, but this is unknown for virus and parasites. Consequently, the Panel believes that the probability of virus and parasites in fishmeal not being inactivated by heat treatment equals that of virus in Category 3 material. For bacteria, the probability may be higher in fishmeal than in Category 3 material

6.3.4.2. Fish oil

The oil produced by a fishmeal-oil process may in practice be separated from the protein fraction before the mixture has reached a sufficient heat treatment. It may be desirable to have a more gentle treatment of fish oil to protect quality. Adequate heating to obtain sufficient inactivation of fish pathogens in the oil fraction is important, also because there is no species restriction in the use of pure (protein free) fish oil.

Microorganisms in pure fish oil can survive but not grow without addition of water. Since water and oil are not mixable, microbial activity always take place at oil-water phase boundaries. For most of the microorganisms in this risk assessment, we are not aware of any information as to their occurrence in the fat fraction of fish material. In the Nofima/NVI report, the authors estimated the concentration of IPN virus in the fat fraction of virus containing fish tissue homogenate to be 1 $\log_{10}$ less than that of the water soluble fraction. Fat is known to offer bacteria a better protection against heat, but this is unknown for virus. Consequently, the Panel believes that the probability of virus in oil not being inactivated by heat treatment equals that of virus in Category 3 material. For bacteria, the probability may be higher in oil than in Category 3 material

Table 4: Summary of hazard characterization

Pathogen	**Susceptible species**	**Prevalence in susceptible species in Norwegian aquaculture**	**Anticipated effect of heating Category 3 material from Norwegian farmed fish 76 ºC / 20 min**	**Anticipated effect of heating Category 3 material from Norwegian farmed fish 85 ºC / 25 min**	**Anticipated effect of the two heat treatment methods on fishmeal of Category 3 material from Norwegian farmed fish**	**Anticipated effect of the two heat treatment methods on fish oil of Category 3 material from Norwegian farmed fish**	**Anticipated effect on the two heat treating methods by silaging (pH 4)**
Infectious hematopoietic necrosis virus (IHNV)	Salmonids. Atlantic salmon is particularly susceptible	Not present	Complete inactivation anticipated	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	Low pH will rapidly inactivate virus
Viral hemorrhagisk septikemi virus (VHSV)	Salmonids, and other marine and fresh water fish species	Only one reported outbreak during the last decades (2007-8)	Complete inactivation anticipated	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	Little or none
Infectious salmon anemia virus (ISAV)	Salmon, possibly salmonids	Low	Complete inactivation anticipated	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	Low pH will inactivate virus given enough time
Nodavirus	Marine species	Low	Complete inactivation anticipated	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	None
Salmon pancreas disease virus (SPDV) also called Salmonoid alpha virus (SAV)	Atlantic salmon, rainbow trout, and several other fish species	Moderate in endemic area and very low to low outside endemic area.	Complete inactivation anticipated	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	Little or none
Infectious pancreatic necrosis virus (IPN virus)	All farmed fish species in Norway	High	Estimated reduction in IPN virus infectivity in fish tissue homogenate approx- 3 $\log_{10}$.	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	None
Piscine reovirus (PRV)	Salmonids	Very high	Data missing	Data missing	Data missing	Data missing	Data missing

Pathogen	**Susceptible species**	**Prevalence in susceptible species in Norwegian aquaculture**	**Anticipated effect of heating Category 3 material from Norwegian farmed fish 76 ºC / 20 min**	**Anticipated effect of heating Category 3 material from Norwegian farmed fish 85 ºC / 25 min**	**Anticipated effect of the two heat treatment methods on fishmeal of Category 3 material from Norwegian farmed fish**	**Anticipated effect of the two heat treatment methods on fish oil of Category 3 material from Norwegian farmed fish**	**Anticipated effect on the two heat treating methods by silaging (pH 4)**
Renibacterium salmoninarum	Salmonids	Very low	Complete inactivation anticipated in culture medium. Possible protective effect of high fat content not known	Complete inactivation anticipated	Same as or lower than on Category 3 material	Lower than on Category 3 material	Increased bactericidal effect
Aeromonas salmonicida subsp. *salmonicida*	Salmonids and other species	Very low	Complete inactivation anticipated.	Complete inactivation anticipated	Same as or lower than on Category 3 material	Lower than on Category 3 material	Increased bactericidal effect
Francisella sp.	Atlantic cod *)	Moderate to high	Data missing, but complete inactivation anticipated.	Data missing, but complete inactivation anticipated.	Same as or lower than on Category 3 material	Lower than on Category 3 material	Data missing
Vibrio(L.) anguillarum	Many fish in the marine environment	Moderate	Complete inactivation anticipated	Complete inactivation anticipated	Same as or lower than on Category 3 material	Lower than on Category 3 material	Data missing
Moritella viscosa	Salmonids	High	Data missing, but complete inactivation anticipated.	Data missing, but complete inactivation anticipated.	Same as or lower than on Category 3 material	Lower than on Category 3 material	Data missing
Lactococcus garviae	Cultured freshwater and marine fish	Not present	Complete inactivation anticipated in culture medium. Possible protective effect of high fat content not known	Complete inactivation anticipated	Same as or lower than on Category 3 material	Lower than on Category 3 material	Little or none

Pathogen	**Susceptible species**	**Prevalence in susceptible species in Norwegian aquaculture**	**Anticipated effect of heating Category 3 material from Norwegian farmed fish 76 ºC / 20 min**	**Anticipated effect of heating Category 3 material from Norwegian farmed fish 85 ºC / 25 min**	**Anticipated effect of the two heat treatment methods on fishmeal of Category 3 material from Norwegian farmed fish**	**Anticipated effect of the two heat treatment methods on fish oil of Category 3 material from Norwegian farmed fish**	**Anticipated effect on the two heat treating methods by silaging (pH 4)**
Yesinia ruckeri	Salmonids	Low	Complete inactivation anticipated in culture medium. Possible protective effect of high fat content not known	Complete inactivation anticipated	Same as or lower than on Category 3 material	Lower than on Category 3 material	Increased bactericidal effect
Flavobacterium phsychrophilum	Rainbow trout and occasionally salmon	Low – moderate	Complete inactivation anticipated in culture medium. Possible protective effect of high fat content not known	Complete inactivation anticipated	Same as or lower than on Category 3 material	Lower than on Category 3 material	Data missing
Gyrodactylus salaris	Salmonids (in fresh water)	Not present	Complete inactivation anticipated	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	Little or none
Lepeophtheirus salmonis (Salmon louse)	Salmonids (in salt water)	High	Complete inactivation anticipated	Complete inactivation anticipated	Same as on Category 3 material	Same as on Category 3 material	Little or none

*) In Norway, only Atlantic cod is considered susceptible. However in Chile, some strains have been reported to cause disease in salmon.

6.4. Risk characterization

6.4.1. Virus

The Panel has no knowledge of any data regarding the effect of heat inactivation or acidification of PRV. PRV is very widespread in Norwegian salmon farming. Lacking this information, the Panel has chosen to anticipate that the PRV is not more heat and acid resistant than IPN, which is the most resistant of those that have been assessed. This may prove to be wrong when relevant information becomes available, and this uncertainty should be kept in mind when reading this risk characterization.

Infectious pancreatic necrosis is often referred to as the biggest health problem in Norwegian aquaculture. The IPN virus can infect all farmed fish species in Norway, and is widely spread in Norwegian aquaculture. The infective dose may be low. IPN virus is assumed to be the most heat resistant of the viruses included in this risk assessment.

Based on the data from the Nofima/NVI report, estimated reduction in IPN virus infectivity at 76 ºC for 20 min in fish tissue homogenate at pH 4 or pH 7 is approximately 3 $\log_{10}$. The IPN virus titres in kidneys of healthy, persistently infected fish have been estimated to 10^2 to 10^4 $TCID_{50}$ g–1 tissue in Atlantic salmon. IPN virus will be present in other tissues than kidney, but supposedly at a lower titre. Consequently, there is a low probability of IPN virus being present in ABP category 3 materials after heat treatment of at 76°C for 20 minutes. Silaging (pH equal to 4 or below) of the ABP category 3 materials will have little or no effect on the outcome of the heat treatment.

Furthermore, the Panel cannot exclude the possibility that the fraction of viruses surviving a suboptimal heat treatment may be more heat resistant than the majority of the virus population. If so, the probability may increase over time if these viruses are recirculated back to the fish farm environment.

The Panel anticipates that the effect of heat treatment on virus in fish oil or fishmeal equals that of ABP category 3 materials. In the Nofima report, the authors estimated the concentration of IPN virus in the fat fraction of fish tissue homogenate to be 1 $\log_{10}$ less than that of the water soluble fraction. Consequently, the probability of IPN virus being present after heat treatment at 76°C for 20 minutes will be lower in fish oil than in ABP category 3 materials and fishmeal, but it will not be negligible.

In the Nofima report, a 3 $\log_{10}$ reduction of IPN virus in fish tissue homogenate was observed after 2.2 min at 85 ºC. Consequently, heat treatment at 85°C for 25 minutes will most probably inactivate all IPN virus in ABP category 3 materials, and fishmeal and fish oil thereof.

Due to the combination of low prevalence and low heat resistance, the probability of the other assessed viruses being present after heat treatment of ABP category 3 materials, and fishmeal or fish oil thereof, is considered to be negligible both at 76°C for 20 minutes and at 85°C for 25 minutes.

6.4.2. Bacteria

Information on heat and acid tolerance is very limited or missing for some of the bacteria assessed. However, the Panel has chosen to anticipate that these bacteria are less heat and acid resistant than *R. salmoninarum* and *L. garvieae*, which are the most resistant of those that have been assessed. This may prove to be wrong when relevant information becomes available, and this uncertainty should be kept in mind when reading this risk characterization.

Data from experiments with *R. salmoninarum* and *L. garvieae* in culture medium indicate a sufficient reduction at 76°C for 20 minutes in this matrix. The Panel is not familiar with data from experiments in matrices equalling ABP, category 3, e.g. with a high fat content which may contribute to higher heat tolerance. However, based on the very low prevalence of *R. salmoninarum* and that *L. garvieae* is not present in Norway, the Panel anticipates the probability of these bacteria being present following heat treatment of ABP category 3 materials at 76°C for 20 minutes to be negligible. The other bacteria assessed are less heat resistant, and will most probably not survive in ABP category 3 materials after heat treatment at 76°C for 20 minutes.

For all the bacteria assessed, the probability of being present following heat treatment of ABP category 3 materials at 85°C for 25 minutes is negligible.

Lowering the pH may trigger a higher heat tolerance in bacteria. However, it is more probable that a pH of 4 and lower, especially when obtained by using organic acids, will have a bactericidal effect. For some of the species assessed, it has been reported that low pH has a bactericidal effect, whereas data is missing for the others. The Panel anticipates that for most of the bacteria assessed, a combination of silaging and heat treatment of ABP category 3 materials is expected to give a higher bactericidal effect than heat treatment alone. One exception is *L. garvieae* which is more tolerant to low pH and consequently less affected by silaging.

Both the high fat content in fish oil and the low water activity in fishmeal may contribute to a higher heat tolerance for bacteria, but the Panel is not familiar with data on such possible effects on fish bacteria. In addition, the Panel lacks information on the probability of any the bacteria being present in the oil. Consequently, the Panel does not have sufficient information to anticipate the probability after heat treatment of fish oil at neither 76°C for 20 minute nor 85°C for 25 minutes. Also, the Panel does not have sufficient information to anticipate the probability after heat treatment of fishmeal at 76°C for 20 minutes. However, considering the heat tolerance observed in other matrices, the probability of presence after heat treatment of fishmeal at 85°C for 25 minutes is considered negligible for all bacteria assessed.

6.4.3. Parasites

Both the assessed parasites have a low tolerance for heat. Therefore, the probability of presence after heat treatment of both ABP category 3 materials, fish oil and fishmeal at both 76°C for 20 minutes and 85°C for 25 minutes is considered negligible for these parasites. Although there is a lack of information on the effect of lowering the pH to 4 or below with organic acids, the Panel anticipates that this will most probably also kill the parasites.

6.4.4. The use of processed salmon oil in salmon feed

Based on the assessment above, fish oil from salmon category 3 materials may still contain IPN virus, and possible also PRV, after heat treatment at 76°C for 20 minutes, but not at 85°C for 25 minutes. IPN virus can infect all farmed fish species in Norway, and the infection dose may be low. PRV can infect salmonids.

The presence of bacterial pathogens after heat treatment at 76°C for 20 minutes or 85°C for 25 minutes cannot be anticipated due to lack of information.

6.4.5. IPN virus as suitable indicator for sufficient inactivation of fish pathogens

The IPN virus appears to be more heat resistant than the other fish pathogens assessed. This should make IPN virus a suitable indicator for testing methods for heat inactivation. However, this is based on the assumption that PRV is not more heat and acid resistant than IPN virus. At present time, the Panel has no knowledge of any data regarding the effect of heat

inactivation. Consequently, this assumption may prove to be wrong when relevant information becomes available,

In the Nofima/NVI report, a cell culture method was used to detect surviving virus after heat treatment, as real-time PCR and classical PCR methods were rejected due to the lack of correlation that was found between positive PCR and viable virus as measured by cell culture isolation. However the use of a cell culture method for IPN virus as indicator organism has some disadvantages. One is that inactivation methods can only be tested on a limited range of natural matrices. Secondly, few laboratories will have facilities for performing cell culture tests. Consequently, the Panel advices that methods more suitable for the detection of viable IPN virus should be established, e.g. determining the presence of an intact genome by pre-PCR sample treatment that determine the integrity of the viral capsid prior to extraction of nucleic acids and subsequent RT-PCR.

In general, detection of viral nucleic acids by PCR methods is relatively straightforward, while detection of infectious virus particles is technically more demanding. PCR methods, and real-time PCR methods specifically, only detect a small part of the viral genome, and there may not be identity between a positive PCR result and infectious virus. The information that is often required in risk assessments is the number of infective viral particles. There are different approaches that are used to overcome this. For environmental samples tested for infectious virus by PCR two approaches has been used, i) determining the presence of an intact genome by direct RT-PCR, or ii) pre-PCR sample treatment that determine the integrity of the viral capsid prior to extraction of nucleic acids (Rodriguez et al. 2009). Damage to the viral capsid may result in the loss of protection of the viral genome and thus its ability to replicate. The detection of an intact genome is therefore an indication that the virus capsid is still intact. Indication of an intact virus genome may be found by amplification of the 5' non-translated region of the viral genome (RNA viruses only), and the analysis of a large portion of the viral genome. Pre-PCR sample treatments with the potential to discriminate between infectious viruses and non-infectious virus include protease and RNase sample pre-treatment, or immunocapture/cell culture attachment of the virus from the sample, prior to extraction of nucleic acids (Rodriguez et al., 2009).

The Panel suggests that NFSA also considers using a bacterial indicator. Bacteria have several stress protection mechanisms not shared by virus. Various mechanisms, resulting in higher temporary tolerance against stresses like heat and acid, can be activated in different environments, e.g. different matrices. Consequently, a virus may not be a representative indicator for bacteria in all test situations.

7. Data gaps

In general, heat resistance of psychrotrophic bacterial pathogens of fish has not been well examined. D- and z- values are determined for some of the fish pathogens, but many values are missing, making exact calculations needed to estimate inactivation at other temperatures impossible. Furthermore, the possible influence of the matrix in which the pathogenic organism is located when heat treated is often unknown.

In particular, information on the effect of heat or acidification of Piscine Reovirus (PRV) in any kind of matrix is missing. The same is the case for *Francisella noatunensis* subsp. *noatunensis* and *Moritella viscosa.*

Information on the presence of fish pathogens in fish oil is lacking, as well as information on the effect of heat inactivation on fish pathogens in fish oil.

8. Answer to the terms of reference

Piscine reovirus (PRV) is very widespread in Norwegian salmon farming. The Panel has no knowledge of any data regarding the effect of heat inactivation or acidification of PRV. Lacking this information, the Panel has chosen to anticipate that the PRV is not more heat and acid resistant than IPN virus, which is the most resistant of those that have been assessed.

Likewise, information on heat and acid tolerance is also very limited or missing for some of the bacteria assessed. Based on general information on psychrotrophic bacteria, the Panel has chosen to anticipate that these bacteria are less heat and acid resistant than *R. salmoninarum* and *L. garvieae*, which are the most resistant of those that have been assessed.

These presumptions may prove to be wrong when relevant information becomes available, and this uncertainty should be kept in mind when reading the answers to the terms of reference.

1. To which risks, in terms of listed diseases and infectious pancreatic necrosis (IPN) virus, will fish be exposed by use of untreated animal by-products (ABP), category 3?

Based on the prevalence of the organisms and/or the disease in Norwegian fish farming, the Panel finds that:

- The probability of exposure to Piscine reovirus (PRV) may be very high.
- The probability of exposure to Infectious pancreatic necrosisvirus (IPN virus), *Francisella* sp., *Vibrio anguillarum,* and *Lepeophtheirus salmonis* (Salmon louse) is moderate to high.
- The probability of exposure to *Flavobacterium phsychrophilum* is low to moderate.
- The probability of exposure to Salmon pancreas disease virus (SPDV) is moderate in endemic area and very low to low outside endemic area.
- The probability of exposure to Infectious salmon anemia virus, Nodavirus, *Renibacterium salmoninarum, Aeromonas salmonicida* subsp. *salmonicida, Yesinia ruckeri,* is very low to low.
- The probability of exposure to Infectious hematopoietic necrosis virus (IHNV), Viral hemorrhagic septicemia virus (VHSV), *Lactococcus garviae* and *Gyrodactylus salaris* is negligible.

For each pathogen, the probability relates to the ABP, category 3 material from the susceptible species.

2. Which risks to fish health will be presented by ABP category 3 materials following heat treatment at 76°C for 20 minutes or 85°C for 25 minutes?

<u>76°C for 20 minutes</u>

The probability of presence of all viruses except IPN virus and possibly also PRV, all bacteria, and all parasites included in this assessment is considered to be negligible following heat treatment of ABP category 3 materials at 76°C for 20 minutes.

The probability of IPN virus, and possibly also PRV, being present in ABP category 3 materials after heat treatment of at 76°C for 20 minutes is considered to be low.

Furthermore, the Panel cannot exclude the possibility that the fraction of viruses surviving a suboptimal heat treatment may be more heat resistant than the majority of the virus population. Consequently, the probability may increase over time if these viruses are recirculated back to the fish farm environment.

85°C for 25 minutes

Following heat treatment of ABP category 3 materials at 85°C for 25 minutes, the Panel anticipates that the probability of presence of all microorganisms assessed is negligible.

3. What are the risks to fish health presented by fish oil or fishmeal originating from ABP category 3 materials after heat treatment at 76°C for 20 minutes or 85°C for 25 minutes?

When answering this question, the Panel presumes that the fish oil and fishmeal has not been exposed to heat treatment with higher temperatures prior in the production process.

76°C for 20 minutes

The probability of presence after heat treatment of fish oil at 76°C for 20 minutes is considered very low for infectious pancreatic necrosis (IPN) virus and possible also for PRV, and negligible for all other viruses assessed.

The probability of presence after heat treatment of fishmeal at 76°C for 20 minutes is low for infectious pancreatic necrosis (IPN) virus, and possibly also PRV, and negligible for all other viruses assessed.

The Panel does not have sufficient information to anticipate the probability of presence of bacteria after heat treatment of fish oil and fishmeal at 76°C for 20 minutes.

The probability of presence after heat treatment of both fish oil and fishmeal at 76°C for 20 minutes is negligible for all parasites assessed.

85°C for 25 minutes

The probability of presence after heat treatment of both fish oil and fishmeal at 85°C for 25 minutes is negligible for all viruses and parasites assessed.

Considering the heat tolerance of the assessed bacteria in other matrices, the Panel assesses that the probability of presence after heat treatment of fishmeal at 85°C for 25 minutes is negligible.

The Panel does not have sufficient information to anticipate the probability of presence of the bacteria after heat treatment of fish oil at 85°C for 25 minutes.

4. What are the risks of infection presented by using processed salmon oil in salmon feed?

The probability of presence after heat treatment of fish oil at 76°C for 20 minutes is considered very low for infectious pancreatic necrosis (IPN) virus, and possibly also PRV, and negligible for all other viruses and parasites assessed. The Panel does not have sufficient information to anticipate the probability of presence of bacteria. IPN virus can infect all farmed fish species in Norway, and the infection dose may be low. PRV can infect salmonids.

The probability of presence after heat treatment of both fish oil and fishmeal at 85°C for 25 minutes is negligible for all viruses and parasites assessed. The Panel does not have sufficient information to anticipate the probability of presence of bacteria.

5. Is documentation of evidence of inactivation of the IPN virus a suitable indicator for sufficient inactivation of fish pathogens?

The IPN virus appears to be more heat resistant than the other fish pathogens assessed. This should make IPN virus a suitable indicator for testing methods for heat inactivation. However, the Panel advices that methods more suitable for the detection of viable IPN virus should be established, e.g. determining the presence of an intact genome by direct RT-PCR, or pre-PCR sample treatment that determine the integrity of the viral capsid prior to extraction of nucleic acids

Furthermore, the Panel suggests that NFSA also considers using a bacterial indicator. Bacteria have several stress protection mechanisms not shared by virus. Consequently, a virus may not be a representative indicator for bacteria in all test situations.

6. To what extent will silaging (pH equal to 4 or below) of by-products affect the answers given to the questions above?

The Panel anticipates that silaging (pH equal to 4 or below) of ABP category 3 materials using formic acid or another organic acid will inactivate IHNV and ISAV, but have little or no effect on heat treatment on the other viruses assessed.

Furthermore, the Panel anticipates that silaging of ABP category 3 materials as described will have a bactericidal effect on most of the bacteria assessed and will most probably also kill the parasites. This effect will come in addition to the effect of heat treatment.

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