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Norwegian Scientific Committee for Food and Environment

VKM Report 2023: 8

Risk assessment of Aquaterra® oil for its intended use as ingredient in fish feed

Scientific Opinion of the Norwegian Scientific Committee for Food and Environment

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of six VKM committee members and two members of the VKM staff. Two referees commented on and reviewed the draft opinion. An appointed group consisting of members from the VKM Panel on Genetically Modified Organisms complemented by a member of the VKM Panel on Animal Feed, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or as part of the approval group.

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VKM would like to thank the referees Jan Willy Pedersen (Danish National Food Institute) and Ragnar Ludvig Olsen (Norwegian College of Fishery Science, The Arctic University of Norway - UiT), for their valuable comments through critical review of the draft opinion. VKM emphasises that the referees are not responsible for the content of the final opinion. In accordance with VKM's routines for approval of a risk assessment (VKM, 2018), VKM received the referees comments before final approval and publication of the report.

Competence of VKM experts

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

Table of contents

Summary

The Norwegian Scientific Committee for food an Environment (VKM) has assessed an application for authorisation of refined oilseed rape oil (Aquaterra®) derived from genetically modified oilseed rape line NS-B50027-4 for exclusive use as an ingredient in fish feed in Norway. NS-B50027-4 is also named DHA-canola. This report uses the term oilseed rape.

NS-B50027-4 produces omega-3 long-chain (≥C20) polyunsaturated fatty acids (omega-3 LC-PUFAs) in its seeds, with a high level of docosahexaenoic acid (DHA) and a small amount of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA). Aquaterra® also contains a significant level of alpha-Linolenic acid (ALA). Whereas ALA can be derived from plants, the primary producers of EPA and DHA are mainly marine microalgae. EPA and DHA are concentrated in the food chain to fish in the oceans and are often referred to as marine omega-3 fatty acids. NS-B50027-4 was developed as an alternative land-based source of marine fatty acids, mainly DHA.

NS-B50027-4 was genetically modified to express seven transgenes derived from yeasts and marine microalgae that encode the enzymes necessary for the biosynthesis of omega-3 long-chain polyunsaturated fatty acids. In addition, an eighth gene, *pat*, was inserted as a marker for selection purposes during development. The *pat* gene encodes the enzyme phosphinothricin N-acetyltransferase (PAT) conferring tolerance to glufosinate-ammonium herbicides. Equally to conventional refined oilseed rape oils any residues levels of proteins, including the introduced enzymes, will be negligible in the Aquaterra® oil.

The risk assessment of Aquaterra® was conducted in accordance with the guidance for risk assessment of derived food and feed from genetically modified plants as described by the European Food Safety Authority (EFSA, 2011a). The risk assessment is based primarily on scientific documentation provided in the application EFSA-GMO-NL-2019-160, which seeks approval for NS-B50027-4 for all applicable food and feed uses in the European Union (EU). VKM concludes that the provided scientific documentation fulfills the criteria of the EFSA guidance and is adequate for risk assessment.

VKM concludes that the molecular characterisation, comparative, nutritional, toxicological and allergenicity assessments of NS-B50027-4 do not indicate increased risks to animal or human health compared to its conventional counterpart (comparator) or commercial reference varieties. Based on this together with specific analyses of the seed oil fraction and studies, e.g., in fish, VKM therefore concludes that the refined oil Aquaterra®, is equal to conventional oils from oilseed rape except for the altered composition in fatty acids.

VKM concludes there is no increased health risk to fish fed Aquaterra® in feed compared to conventional feeds with oils from other sources, nor is there an indication of increased risk to the environment. Since Aquaterra® is equal to conventional oils from oilseed rape except for the marine omega-3 fatty acids already present in fish feeds, VKM concludes there is no

greater need for health or environmental monitoring of feeds containing Aquaterra® than conventional feeds.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority, Aquaterra®, oilseed rape, canola, omega-3 long-chain (≥C20) polyunsaturated fatty acids, omega-3 LC-PUFAs, fish feed, GMO, genetically modified canola, DHA-canola, NS-B50027-4, genetically modified rapeseed oil.

Sammendrag på norsk

Vitenskapskomiteen for mat og miljø (VKM) har vurdert en søknad om godkjenning av prosessert rapsolje, Aquaterra®, fra genmodifisert raps NS-B50027-4, utelukkende til bruk som ingrediens i fiskefôr i Norge.

NS-B50027-4 produserer langkjedede (≥C20) flerumettede omega-3-fettsyrer (omega-3 LC-PUFA) i frøene, med et høyt nivå av dokosaheksaensyre (DHA) og små mengder eikosapentaensyre (EPA) og dokosapentaensyre (DPA). Aquaterra® inneholder også et betydelig nivå av alfa-linolensyre (ALA). ALA kan utvinnes fra planter, mens de primære produsentene av EPA og DHA hovedsakelig er marine mikroalger. EPA og DHA akkumuleres oppover i næringskjeden, spesielt i fet fisk i havet, og blir ofte referert til som marine omega-3 fettsyrer. Raps NS-B50027-4 ble utviklet som et landbasert alternativ til marine fettsyrer, hovedsakelig DHA.

Raps NS-B50027-4 er genmodifisert til å uttrykke syv transgener avledet fra gjær og marine mikroalger. Transgenene koder for nødvendige enzymer i biosyntesen av de marine omega-3 fettsyrene. Et åttende transgen, *pat*, ble satt inn som seleksjonsmarkør under utviklingen av rapsen. *Pat* genet koder for enzymet fosphinothricin N-acetyltransferase (PAT), som gir toleranse for ugressmidler som er basert på glufosinat-ammonium. I likhet med konvensjonelle prosesserte rapsoljer vil eventuelle rester av proteiner, inkludert de introduserte enzymene, være ubetydelig i oljen Aquaterra®.

VKMs risikovurdering av Aquaterra® ble utført i samsvar med veiledningen fra Den europeiske myndighet for næringsmiddeltrygghet (EFSA) for risikovurdering av genmodifiserte planter til bruk i mat og fôr (EFSA, 2011a). Risikovurderingen er i all hovedsak basert på dokumentasjon fra søknaden 'EFSA-GMO-NL-2019-160', om godkjenning av raps NS-B50027-4 til aktuelle bruksområder innen mat- og fôr i EU. VKM konkluderer at den vitenskapelige dokumentasjonen vedlagt i EFSA-GMO-NL-2019-160, oppfyller kriteriene i EFSAs veiledning og er tilstrekkelig for risikovurderingen.

VKMs konklusjon er at den molekylære karakteriseringen, de komparative, ernæringsmessige og toksikologiske analysene, og vurderingen av allergent potensiale av raps NS-B50027-4, ikke indikerer økt helserisiko for dyr eller mennesker sammenliknet med rapsens konvensjonelle motpart (komparator) eller kommersielle referansesorter. På bakgrunn av dette og av analyser av selve oljen Aquaterra® og fôringsstudier med b.la. fisk, konkluderer VKM at prosessert olje fra raps NS-B50027-4 tilsvarer konvensjonelle rapsoljer, med unntak av de tilsiktede endringene i fettsyrer.

VKMs konklusjon er at fiskefôr som inneholder Aquaterra® ikke gir økt helserisiko for fisk sammenliknet med konvensjonelt fiskefôr med oljer fra andre kilder. Det er heller ingen indikasjon på økt miljørisiko ved bruk av Aquaterra® i fôr til fisk fremfor oljer fra andre kilder. Ettersom Aquaterra® tilsvarer konvensjonelle rapsoljer med unntak av de marine omega-3-fettsyrene, som allerede brukes i fiskefôr, konkluderer VKM at det ikke er noe større behov

for helse- eller miljøovervåking av fôr som inneholder Aquaterra® enn for konvensjonelt fiskefôr. Et utvidet norsk sammendrag er vedlagt rapporten under Appendix 1.

Abbreviations and glossary

Abbreviations

BLAST	Basic Local Alignment Search Tool.
DNA	Deoxyribonucleic acid.
DHA	Docosahexaenoic acid.
EFSA	European Food Safety Authority.
FASTA	Fast Alignment Search Tool – All.
GM	Genetically modified.
GMO	Genetically modified organism.
KASP	Kompetitive Allele Specific PCR.
LC-MRM-MS	Liquid chromatography/multiple reaction monitoring/mass spectrometry.
Omega-3 LC-PUFAs	Omega-3 long chain polyunsaturated fatty acids.
OECD	Organisation for Economic Co-operation and Development.
ORF	Open reading frame.
PAT	Phosphinothricin N-acetyltransferase.
PCR	Polymerase chain reaction.
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis.
T-DNA	Transfer DNA.
VKM	Norwegian Scientific Committee for Food and Environment

Glossary

Comparator	The unmodified conventional counterpart used as control to the genetically modified organism, to detect characteristical differences resulting from the genetic modifications.
DHA-Canola	Oilseed rape genetically modified to produce the omega-3 long chain polyunsaturated fatty acid docosahexaenoic acid. In this case oilseed rape NS-B50027-4.
EFSA guidance	Refers to one or several documents published by the European Food Safety Authority (EFSA) that outline specific approaches and considerations for risk assessment.
Elongases	Elongases are enzymes that catalyses carbon (C) chain extensions of organic molecules, especially fatty acids. E.g., the Δ5-elongase enzyme which is involved in the conversion of eicosapentaenoic acid (EPA, 20:5) to docosapentaenoic acid (DPA, 22:5).
Fatty acids	Fatty acids are the building blocks of the fat in our bodies and in the food we eat. Fatty acids have many important biological functions. Fatty acids are classified by length, by saturation vs unsaturation, by even vs odd carbon (C) content, and by linear vs branched molecular structures. Saturated fatty acids have no C=C double bonds whereas unsaturated fatty acids have one or more C=C double bonds
Fatty acid desaturases	Enzymes that remove two hydrogen atoms from a fatty acid, creating a carbon/carbon double bond. Classified as either: 1) Delta (Δ) - indicating that the double bond is created at a fixed position from the carboxyl end of a fatty acid chain. For instance, Δ9 desaturase creates a double bond between the ninth and tenth carbon atom from the carboxyl end. 2) Omega (ω) - indicating the double bond is created at a fixed position from the methyl end of a fatty acid chain. For instance, ω3 desaturase creates a double bond between the third and fourth carbon atom from the methyl end, i.e., it creates an omega-3 fatty acid.

Genetic modification	The process of inserting novel DNA/genes from the same or foreign species or deleting genes. Common to all is the use of recombinant DNA technology.
GM crops	Genetically modified crops - cultivated plants whose genetic characteristics have been changed usually by the insertion of modified and recombined genes from the same or from a different species using the techniques of genetic engineering.
Herbicide	A chemical or other substance that is toxic to plants, used to destroy unwanted vegetation, e.g. weeds on agricultural land.
ORF	Open reading frame, ORFs are roughly defined as spans of DNA sequence between start and stop codons, that encode an amino acid sequence (protein) according to the genetic code.
Post-market monitoring	A predefined strategy to monitor for possible adverse effects to human or animal health and the environment.
Transgene	A gene that is transferred from an organism of one species to an organism of another species by genetic engineering.
Unintended effect	Predicted or unpredicted effects from the genetic modification occurring at the genetic, organismal and/or environmental level.
Vector	A vehicle, often a virus or a plasmid carrying desired DNA into a host cell and can also assist in multiplying or expressing the insert.

Background as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority is the approval authority for applications of products derived from GMOs for use as food and feed. Norway has not yet incorporated the EU regulations on genetically modified food and feed but has national regulations under the Food Act which contains the most relevant elements from the EU regulations when it comes to i.a., risk assessment, approval, and labelling. The Food Act sets the framework for the Norwegian Food Safety Authority's assessment of the applications. Regulation 7 November 2002 no. 1290 on feed (the feed regulation), regulates genetically modified feed. The objects clause, cf. § 1, states that feed must be safe and have no injurious to human or animal health or make food from animals unfit for consumption. Nor should the feed have any harmful effects on the environment.

On 7 June 2022, the Norwegian Food Safety Authority received an application from Nufarm B.V. in the Netherlands on behalf of Nuseed Nutritional Australia Pty Ltd., regarding the approval of the genetically modified oil Aquaterra® derived from the genetically modified oilseed rape with unique code NS-B5ØØ27-4. The application concerns import and use of the Aquaterra® oil in fish feed.

NS-B5ØØ27-4 was genetically modified by *Agrobacterium tumefaciens*-mediated transformation, where seven genes from species of yeast and marine microalgae were inserted into the plant's genome. The genes encode enzymes that are involved in the biosynthesis of fatty acids that lead to the production of several long-chained, omega-3 fatty acids (≥C20) in the seeds of the plant. These long-chained fatty acids are usually extracted from marine organisms. It is particularly the end product docosahexaenoic acid (DHA) that accumulates in large quantities in the seeds, but also several other long-chained fatty acids, i.a. eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA).

The oilseed rape also contains a gene that encodes an enzyme that confers tolerance to herbicides with glufosinate ammonium. The gene was initially used as a selection marker during the transformation process, but some farmers who grow the oilseed rape make use of this characteristic in their weed control after being trained by Nuseed.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) has requested the Norwegian Scientific Committee for Food and Environment (VKM) to perform a scientific risk assessment of Aquaterra® oil for its intended use as an ingredient in fish feed. VKM is tasked with assessing possible negative health effects in fish fed Aquaterra® oil. The risk assessment shall apply to the use of Aquaterra® oil as an ingredient in fish feed with a particular focus on such feed to species of salmonids (family *Salmonidae*), including Atlantic Salmon (*Salmo salar*).

According to the Feed Regulation § 1 (FOR-2002-11-07-1290), feed should not have adverse effects on the environment. If VKM finds it necessary, possible unintended effects on the environment by using fish feed with Aquaterra® oil, should be assessed. If not relevant, VKM shall state the reason for this.

Based on the risk assessment, the Norwegian Food Safety Authority asks VKM to consider the need for a monitoring plan to reveal unintended effects on health and environment.

Methodology and Data

Data and information gathering

Key data used in this report for the risk assessment of Aquaterra®, was the scientific documentation submitted by the applicant to the European Food Safety Authority (EFSA) in connection with an application (EFSA-GMO-NL-2019-160) for approval of the genetically modified oilseed rape NS-B50027-4 for food and feed uses in the EU. The VKM GMO Panel previously assessed this documentation in connection with the EFSA scientific hearing of application EFSA-GMO-NL-2019-160 which ended on 20.08.2022.

Additional scientific literature referred to in the report were acquired by individual searches made by project members and by a librarian at the National Institute of Public Health (NIPH), Norway.

Literature search and selection

Databases used by NIPH were Ovid MEDLINE(R), Embase, Web of Science and CAB Abstracts. The search strategy and search terms are available in Appendix 2.

Total records identified across the four databases, after removal of duplicates, were 79 results relating to the applied search terms. These were imported into EndNote™ 20 and screened for their relevance by two project members. Only five of the 79 publications were found relevant and included in the risk assessment of Aquaterra® as ingredient in fish feed.

Remaining references used in the report were acquired through individual searches made by project members and included where appropriate in the different chapters of the report. E.g., in chapter 1.1 "Nutritional requirements of fish and use of oil (lipids) in aquaculture (salmon)", articles were acquired via «google scholar» with the search words «DHA canola oil and salmon nutrition».

Assessment

This report is divided into two main parts.

Part I

Briefly introduces the intended use of refined oilseed rape oil, Aquaterra®, as an alternative source of important nutrient fatty acids for fish in aquaculture. Next follows an abbreviated assessment of potential health risk to fish, environmental risk, and whether there is a need for health or environmental monitoring with the use of Aquaterra® in fish feed. Finally, Part I concludes with answers to the Terms of Reference (ToR) defined by the Norwegian Food Safety Authority (NFSA).

Part II,

Describes the risk assessment by VKM of essential scientific documentation used to derive the conclusions answering the Terms of Reference. The scientific areas covered by the risk assessment include molecular characterisation, compositional analyses, nutritional analyses, toxicology and allergenicity testing relating to the oilseed rape NS-B50027-4 plant and its derived products. The risk assessment was conducted in accordance with the guidance for risk assessment of derived food and feed from genetically modified plants as described by the European Food Safety Authority (EFSA, 2011a).

Part I

Introduction

The Norwegian Scientific Committee for food an Environment (VKM) has assessed an application for authorisation made by Nufarm B. V. on behalf of Nuseed Nutritional Australia Pty Ltd. to seek approval for refined oilseed rape oil (Aquaterra®) derived from the genetically modified oilseed rape line NS-B50027-4 for use in fish feed in Norway.

NS-B50027-4 produces omega-3 long-chain (≥C20) polyunsaturated fatty acids (omega-3 LC-PUFAs) in seed oil, with a high level of docosahexaenoic acid (DHA), a small amount of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA). The oil fraction also contains a significant level of alpha-Linolenic acid (ALA), an essential omega-3 fatty acid commonly found in vegetable oils, seeds, and nuts.

Omega-3 oils containing omega-3 LC-PUFAs are important nutritional components for both humans and animals and are indicated as beneficial for several health parameters e.g., cognitive development, cardiovascular condition, inflammatory and immune responses, hormone regulation and more. Whereas alpha-Linolenic acid, a medium chain omega-3 fatty acid, can be derived from plants, the primary producers of EPA and DHA are marine microalgae (phytoplankton). EPA and DHA are concentrated in the food chain of marine fish, especially oily fish species e.g., herring, sardines, salmon and trout. EPA and DHA are therefore often referred to as marine omega-3 fatty acids.

NS-B50027-4 was developed as an alternative land-based source of DHA and to a lesser degree EPA and DPA.

The genetic modifications resulting in the oilseed rape line NS-B50027-4 inserted two new genes from two different yeasts and five new genes from three different marine micro algae into the plant genome. These genes encode the enzymes (desaturases and elongases) necessary for the biosynthesis of the marine omega-3 fatty acids. In addition, an eighth gene, *pat*, was inserted as a marker for selection purposes during development. *Pat* encodes the enzyme phosphinothricin N-acetyltransferase (PAT) conferring tolerance to glufosinate-ammonium herbicides. Equally to conventional refined oilseed rape oils any residues levels of proteins, including the introduced enzymes, will be negligible in the Aquaterra® oil.

The risk assessment of Aquaterra® was performed by a VKM-appointed project group consisting of experts within the fields of fish feed/fish nutrition, fish health, molecular biology, plant physiology, genetics, allergenicity, toxicology and genetically modified organisms (GMOs). The assessment was conducted in accordance with the guidance for risk assessment of derived food and feed from genetically modified plants as described by the European Food Safety Authority (EFSA, 2011a). The report includes previous considerations

made by the VKM GMO Panel during the EFSA scientific hearing of application EFSA-GMO-NL-2019-160, which seeks approval for oilseed rape NS-B50027-4 for all applicable food and feed uses in the EU.

The report starts by answering the requested Terms of Reference (ToR) before presenting the scientific areas covered by the risk assessment, that provide the basis for the deduced conclusions. The scientific areas include molecular characterisation, compositional analyses, nutritional analyses, toxicology and allergenicity testing relating to oilseed rape NS-B50027-4 and its derived products.

The report was reviewed by two independent external referees before given final approval by the VKM GMO panel, complemented by a member of the VKM Panel on animal feed.

Terms of Reference (restated)

1. Assessment of possible negative health effects in fish fed the Aquaterra® oil as an ingredient in fish feed with a particular focus on such feed to species of salmonids.

2. Assessment of possible unintended effects to the environment by using fish feed with Aquaterra® oil. If not relevant, VKM shall state the reason for this.

3. Consider the need for a monitoring plan to reveal unintended effects on health and environment.

1 Risk assessment of Aquaterra® as ingredient in fish feed

1.1 Nutritional requirements of fish and use of oil (lipids) in aquaculture (salmon)

The inclusion of lipids in formulated fish diets varies depending on the species and life stage. High-quality protein is expensive, and the fish species farmed in Norway are carnivorous with a low tolerance for carbohydrates, leading to an incentive to use as much energy from oil as possible in fish feed. The amount of oil is therefore usually based on the upper tolerance for neutral lipids for the specific species at each developmental stage. Marine species such as halibut, cod, ballan wrasse, and lumpfish should be given diets with 10 to 20 % lipid, while salmonids may be fed diets with 38% lipid at the final stages before slaughter. Given the volume of farmed Atlantic salmon compared to the other farmed species in Norway, the nutritional assessment of Aquaterra® is largely based on salmon.

Besides a source for energy, lipids also contain fatty acids that are important structurally as well as a substrate for bioactive molecules. Two such fatty acids are EPA and DHA that are essential nutrients for marine fish. However, the requirement for Atlantic salmon has proven difficult to establish (Bou et al., 2017). Growth trials in sea water for shorter periods has suggested the requirement to be quite low (Glencross, 2009), but when examining the whole growth period of farmed salmon in the sea it was found that the specific requirement for EPA and DHA was 10 g EPA & DHA per Kg feed (>2.7 % of total dietary fatty acids) (Rosenlund et al., 2016). However, recent results suggest that 35 g EPA & DHA per kg feed will improve growth and welfare of farmed salmon in the sea (Lutfi et al., 2023). In Atlantic salmon feeds and other aquafeeds, the required level of the essential fatty acids is achieved by a mix of oils as well as fatty acid fractions of meals, essentially fish meals. Fatty acids from fish meals derive mostly from phospholipids and constitute a small part of the meal, however the DHA and EPA concentration of the total fatty acids present is high.

In fish oils the ratio between EPA and DHA is usually around 1, but the Aquaterra® oil has very low EPA levels resulting in a ratio of less than 0.05. When it previously was believed that the most important role of EPA was to serve as a substrate for elongation to produce DHA, there are now several studies supporting the view that EPA has important cellular functions (Lutfi et al., 2023). Oilseed rape oil has a high content of n-6 fatty acids compared to fish oil. High inclusion of n-6 will increase the requirement of EPA (Sissener et al., 2020). The Aquaterra® oil may therefore not fully replace fish oil in Atlantic salmon diets, as it does not meet the requirements of EPA that the fish probably require.

Plant oils, the Aquaterra® oil included, does not contain cholesterol, but phytosterols. Whether Atlantic salmon has a requirement for cholesterol or not is unknown. However, it

has been shown that low dietary levels of cholesterol lead to increased endogenous cholesterol synthesis (Sissener et al., 2018). This is not affected by the absorption of phytosterols from plant oils like oilseed rape oil. No negative effect of dietary levels of phytosterols up to 3000 mg/Kg feed has been detected (Sissener et al., 2017). In a study where salmon were fed a diet with 7.8 % inclusion of Aquaterra® oil, resulting in 739 mg phytosterol per Kg feed, no increased levels of phytosterols in the liver compared to fish fed a fish oil-based diet were found (Ruyter et al., 2019).

1.1.1 Nutritional balancing of fish feed

In fish feed for salmonids, it is common practice to use oilseed rape oil and add fish oil to balance the lipid profile and meet the feed producers target for EPA and DHA. As such, the Aquaterra® oil may contribute to reduce the use of fish oil when replacing conventional oilseed rape oil.

1.1.2 Assessment of potential health risks of Aquaterra® oil to fish in aquaculture

The scientific documentation provided in the main application seeking approval for all food and feed uses of oilseed rape NS-B50027-4 in the EU (EFSA-GMO-NL-2019-160) includes a full compositional analysis including a detailed analysis of the seed oil-fraction. The analysis demonstrates that the oil fraction is equivalent to conventional oilseed rape oils except for the intended changes in fatty acid profiles.

Under the assumption that lipid composition of fish feeds will be equally well balanced irrespective of the origin of the raw materials, there is no reason to expect any detrimental health effects to fish, if partly substituting existing sources of oils with Aquaterra® oil.

1.1.3 Assessment of potential risks of Aquaterra® oil to the environment surrounding fish pens

Fish farming is associated with potential negative impacts to the environment. The type and severity of the environmental effects depend on factors, e.g., production scale, species farmed, site of production, medicinal treatments etc. Negative effects of local nutrient build up in areas with aquaculture is one which is directly linked to fish waste (faeces) and unconsumed feed that fall to the ocean floor.

Given that Aquaterra® oil is composed of fatty acids already present in existing fish feeds it can be deduced that there will be no added environmental risks if Aquaterra® is used as an alternative source of oil in fish feed.

1.1.4 Post-market health and environmental monitoring

Aquaterra® oil is intended for import as a final refined product in line with conventional oilseed rape oils and as an alternative ingredient source for uses in fish feed only, in Norway. Compositional analyses have demonstrated that Aquaterra® is comparable to conventional oilseed rape oils except for the introduced omega-3 LC-PUFAs. These fatty acids are already present in conventional fish feeds. The Aquaterra® oil does not contain detectable levels of the newly expressed proteins required for the DHA synthesis. Nevertheless, these proteins have been investigated for possible detrimental effects to fish health and the environment. No adverse effects were found, indicating no additional need for health or environmental monitoring of feed containing Aquaterra® oil compared to conventional feeds with inclusion of oils from other sources.

Answers to the Terms of Reference – Main conclusions

1. **Assessment of possible negative health effects in fish fed the Aquaterra® oil as an ingredient in fish feed with a particular focus on such feed to species of salmonids.**

Based on the scientific documentation provided in the application, and scientific literature, VKM concludes there is no increased health risk to fish fed Aquaterra® in feed compared to conventional feeds with oils from other sources.

2. **Assessment of possible unintended effects to the environment by using fish feed with Aquaterra® oil. If not relevant, VKM shall state the reason for this.**

The scientific documentation provided in the application demonstrates that the refined Aquaterra® oil is compositionally comparable to conventional oilseed rape oils except for the marine omega-3 fatty acids already in use in fish feeds. VKM therefore concludes there is no indication of an increased risk to the environment with the use of Aquaterra® oil in feeds compared to conventional feeds with oils from other sources.

3. **Consider the need for a monitoring plan to reveal unintended effects on health and environment.**

Since Aquaterra® is equal to conventional oils from oilseed rape except for the marine omega-3 fatty acids already present in fish feeds, VKM concludes there is no greater need for health or environmental monitoring of feeds containing Aquaterra® than conventional feeds.

Part II

2 Risk assessment of genetically modified oilseed rape NS-B5ØØ27-4 for food and feed uses in the EU (application EFSA-GMO-NL-2019-160)

Background

Assigned by the Norwegian Food Safety Authority (NFSA) and the Norwegian Environment Agency (NEA), VKM assesses GMOs and derived products thereof, for which there are sought approval for authorisation to the European market under the Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. VKM is requested to perform assessments for all GMO applications made accessible through the EFSA Document Management System (DMS), where the main focus should be on potential health or environmental risks specific to Norway compared to the EU.

The risk assessments of genetically modified organisms by VKM adhere to the comprehensive EFSA guidance on risk assessment of GMOs for use as food or feed.

In accordance with the assignment from NFSA and NEA, the VKM GMO Panel assessed the application for authorisation of oilseed rape NS-B5ØØ27-4 for all relevant food and feed uses in the EU (application EFSA-GMO-NL-2019-160) during the EFSA scientific hearing in 2022, as part of the (ongoing) risk assessment by EFSA.

Scientific basis for the risk assessment of Aquaterra® in fish feed in Norway

The following chapters describe the scientific basis for the risk assessment of Aquaterra® resulting in the deduced conclusions to the Terms of Reference in Part I. I.e., relevant scientific documentation/data provided in application EFSA-GMO-NL-2019-160 were re-assessed during the risk assessment of Aquaterra® by the appointed project-group. For each scientific topic relevant findings are discussed and concluded.

3 Molecular characterisation

Molecular characterisation is necessary to provide an insight on the genetic material introduced into the genetically modified plants genome and lays ground for the subsequent parts of the safety assessment as outlined in the EFSAs Guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011a). The molecular characterisation provides information on e.g., transformation method, the structure and expression of insert(s), and description and stability of new trait(s).

The main scientific documentation used in the VKM risk-assessment of Aquaterra® was the documentation provided in application EFSA-GMO-NL-2019-160. Additional references used in chapter 3: (FSANZ, 2017); (Petrie et al., 2020), (Colgrave et al., 2019) and (MacIntosh et al., 2021).

3.1 Information related to the genetic modification

3.1.1 Description of the methods and vectors used for the genetic modification

Oilseed rape NS-B5ØØ27-4 was developed through *Agrobacterium tumefaciens* mediated transformation of cotyledonary petioles (leaf of a plant seedling), integrating seven new genes (*Lackl-Δ12D, Picpa-ω3D, Micpu-Δ6D, Pyrco-Δ6E, Pavsa-Δ5D, Pyrco-Δ5E and Pavsa-Δ4D*) into the genome. The genes encode enzymes involved in the biosynthesis of the marine fatty acids (mainly docosahexaenoic acid (DHA)) in the seeds of oilseed rape NS-B5ØØ27-4. An eighth gene, *phosphinothricin N-acetyltransferase* (*pat),* was inserted as a selection marker. The encoded enzyme confers tolerance to glufosinate-ammonium based herbicides.

In brief, Cotyledonary petioles from recipient oilseed rape seedlings (variety AV Jade) were co-cultured with disarmed *Agrobacterium tumefaciens* strain AGL1 containing the binary vector pJP3416_GA7-ModB. Vector pJP3416_GA7-ModB contains elements needed for the transformation of the plant cells, i.e., T-DNA border fragments and the T-DNA (transfer-DNA) with the eight genes and their regulatory sequences.

The oilseed rape variety AV Jade was selected as the recipient line for production of NS-B5ØØ27-4 because, as an elite line, it displayed good transformation efficiency. No helper plasmids or carrier DNA were used in the transformation process.

3.1.2 Source and characterisation of nucleotide acids used for transformation

Oilseed rape variety AV Jade was transformed with binary vector pJP3416_GA7-ModB designed to convert native oleic acid, linoleic acid and alpha-linolenic acid to docosahexaenoic acid (DHA) in the seeds of NS-B5ØØ27-4. The seven introduced genes encoding enzymes involved in fatty acid biosynthesis pathways originate from yeast [Δ12-desaturase (*Lackl-Δ12D* from *Lachancea kluyveri*) and ω3-/Δ15-desaturase (*Picpa-ω3D* from *Pichia pastoris*)], and from marine microalgae [Δ6-desaturase (*Micpu-Δ6D* from *Micromonas pusilla*), Δ6-elongase (*Pyrco-Δ6E* from *Pyramimonas cordata*), Δ5-desaturase (*Pavsa-Δ5D* from *Pavlova salina*), Δ5-elongase (*Pyrco-Δ5E* from *Pyramimonas cordata*) and Δ4-desaturase (*Pavsa-Δ4D* from *Pavlova salina*)]. The sequences were constructed in a T-DNA vector under the control of seed-specific promoters.

A description of the donor organisms for the genes involved in the DHA-biosynthesis pathway:

- *Lachancea kluyveri* (yeast species) is used in food industry for products such as Emmental, Roquefort, Damietta and Greek cheeses, and fermented milk. *L. kluyveri* appears widespread in the environment.
- *Pichia pastoris* (yeast species) is used as a source of proteins for animal feed. It is further used for protein production using recombinant DNA techniques. Many food proteins, pharmaceuticals and enzymes have been expressed in *P. pastoris*, ranging from bacterial proteins to human proteins.
- *Micromonas pusilla* (green algae species) gains energy through photosynthesis and is an important food source for many organisms in aquatic environments, especially for bivalves. *M. pusilla* is used to feed oyster larvae.
- The microalgae *Pyramimonas cordata* plays a key role in the marine food web. *P. cordata* has been used for isolation of phytosterols.
- The microalgae *Pavlova salina* is used to feed oyster larvae to support their growth rate and improve nutritional value.

The last coding sequence within the T-DNA encodes the enzyme phosphinothricin-N-acetyltransferase (PAT) from the bacterium *Streptomyces viridochromogenes* that was used as a selectable marker in the transformation process. *S. viridochromogenes* is a saprophytic, soil-borne microbe, which is not typically pathogenic to animals or humans. *S. viridochromogenes* is not used in the food industry directly, but the *pat* gene has been used to confer glufosinate ammonium-tolerance in food-producing crops for many years.

Additionally, regulatory sequences (seed-specific promoter, enhancer, and terminator) derived from plants (*Linum usitatissimum* (flax), *Arabidopsis thaliana*, *Glycine max* (soybean) and *Nicotiana tabacum* (tobacco)), viruses (*Tobacco mosaic virus* and *Cauliflower mosaic virus*) and bacterium (*Agrobacterium tumefaciens*) were used in the genetic modification of NS-B5ØØ27-4. These regulatory sequences are a very small part of their genome, used for

expression of the inserted genes and are not related to potential adverse effects of these organisms.

The seven DHA-pathway genes and *pat* were chemically synthesized based on the sequences originally identified and characterised from yeast, microalgae, and *S. viridochromogenes,* and were codon optimised for optimal expression in oilseed rape.

3.1.3 History of safe use

The introduced DHA-pathway enzymes are Δ12-desaturase, ω3-/Δ15-desaturase, Δ6-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and Δ4-desaturase. Similar enzymes with the same function as the ones used to develop oilseed rape NS-B5ØØ27-4 are found in yeasts and marine plants that have been consumed as food, used in food production, or used in animal feeds. These organisms are commonly found in the environment and have a history of safe consumption and use in food or feed.

The PAT enzyme is widely used in GM food crops to confer tolerance to herbicides based on glufosinate ammonium. PAT and many transgenic crops that express the PAT enzyme have been assessed by EFSA and considered safe.

3.1.4 Nature and source of vector(s) used including nucleotide sequences intended for insertion

The binary vector pJP3416_GA7-ModB (31,564 bp) used for the transformation contains an 8,052 bp vector backbone and eight expression cassettes between the right and left border of the T-DNA (23,512 bp). Seven of the eight expression cassettes were designed to convert oleic acid to DHA in the seeds of NS-B5ØØ27-4, each encoding one enzyme essential for the pathway. The last expression cassette contains the herbicide tolerance gene (*pat*).

Description of the eight expression cassettes:

Lackl-Δ12D cassette: The *Lackl-Δ12D* gene (coding sequence of the enzyme Δ12-desaturase from yeast *Lachancea kluyveri)* is regulated by the promoter from the seed-specific *conlinin1* gene and terminator from the same *conlinin1* gene. The seed-specific Δ12-desaturase enzyme inserts a double bond at the delta-12 (omega-6) position of oleic acid to give linoleic acid.

Picpa- ω3D cassette: The *Picpa- ω3D* gene (coding sequence of the enzyme Δ15−/ω3-desaturase from yeast *Pichia pastoris)* is regulated by the promoter from the seed-specific conlinin1 *(Linum usitatissimum conlinin1)* gene and terminator from the same *conlinin1* gene. Δ15−/ω3-desaturase enzyme inserts a double bond between the third and fourth carbon from the methyl end (ω end) of a fatty acid. In oilseed rape NS-B5ØØ27-4, this double bond is added to linoleic acid to give alpha-linolenic acid. *Micpu-Δ6D* cassette*:* The *Micpu-Δ6D* gene *(*cloned from microalga *M. pusilla)* is regulated by the seed-specific

conlinin2 *(Linum usitatissimum conlinin2)* gene promoter and terminator from the same gene. The encoded enzyme Δ6-desaturase is required for the conversion of alpha-linolenic acid to stearidonic acid. *Pyrco-Δ6E* cassette: The *Pyrco-Δ6E* gene (coding sequence of the enzyme Δ6-elongase from microalgae *Pyramimonas cordata)* is regulated by the *Arabidopsis thaliana* fatty acid elongase1 gene promoter and terminator from the *lectin* gene from soybean (*Glycine max*). Δ6-elongase is a highly efficient enzyme that converts stearidonic acid to eicosatetraenoic acid.

Pavsa-Δ5D cassette: The *Pavsa-Δ5D* gene (coding sequence of the enzyme Δ5-desaturase from microalgae *Pavlova salina)* is regulated by the seed-specific promoter from the *napin* gene of *Brassica napus*, enhancer from *tobacco mosaic virus* and terminated by the *nos-terminator* from *Agrobacterium tumefaciens.* The Δ5-desaturase enzyme shows very high efficiency in desaturating its substrate, and in oilseed rape NS-B5ØØ27-4, the double bond is added to eicosatetraenoic acid to give EPA.

Pyrco-Δ5E cassette*:* The *Pyrco-Δ5E* gene (coding sequence of the enzyme Δ5-elongase from microalgae *Pyramimonas cordata)* is regulated by embryo-specific *Arabidopsis thaliana* fatty acid elongase1 (FAE1) gene promoter, enhancer from *tobacco mosaic virus* and terminator from the *lectin* gene from soybean*.* The Δ5-elongase enzyme has high efficiency in the conversion of EPA (eicosapentaenoic acid) to DPA (docosapentaenoic acid).

Pavsa-Δ5D cassette: The *Pavsa-Δ5D* gene (coding sequence of the enzyme Δ5-desaturase from microalgae *Pavlova salina)* is regulated by the seed-specific promoter from the *napin* gene of *Brassica napus*, enhancer from *tobacco mosaic virus* and terminated by the *nos-terminator* from *Agrobacterium tumefaciens.* The Δ5-desaturase enzyme shows very high efficiency in desaturating its substrate, and in oilseed rape NS-B5ØØ27-4, the double bond is added to eicosatetraenoic acid to give EPA.

Picpa- ω3D cassette: The *Picpa- ω3D* gene (coding sequence of the enzyme Δ15−/ω3-desaturase from yeast *Pichia pastoris)* is regulated by the promoter from the seed-specific conlinin1 *(Linum usitatissimum conlinin1)* gene and terminated by the same *conlinin1* gene. Δ15−/ω3-desaturase enzyme inserts a double bond between the third and fourth carbon from the methyl end (ω end) of a fatty acid. In NS-B5ØØ27-4, this double bond is added to linoleic acid to give alpha-linolenic acid.

Pavsa-Δ4D cassette: The *Pavsa-Δ4D* gene (coding sequence of the enzyme Δ4-desaturase from microalgae Pavlova salina) is regulated by the seed-specific conlinin2 *(Linum usitatissimum conlinin2)* gene promoter and terminator from the same gene. The enzyme Δ4-desaturase catalyses the Δ4-desaturation of DPA into DHA.

pat cassette: The *pat* gene (coding sequence of the enzyme *phosphinothricin N-acetyltransferase* from the bacterium *Streptomyces viridochromogenes)* is driven by the constitutive *CaMV* (*Cauliflower mosaic virus) 35S* promoter. Transcription is terminated by the polyadenylation signal from the *nos* gene of *Agrobacterium tumefaciens.* The *pat* gene confers tolerance to herbicides containing glufosinate ammonium.

VKM concludes that the applicant has adhered to the recommendations of the EFSAs guidance (EFSA, 2011a). The applicant has provided sufficient and detailed information about methods, nature and source of vectors, vector constructs, transformation process, and transgene constructs in the GM plant.

3.2 Information relating to the GM plant

3.2.1 General description of the trait(s) and characteristics which have been introduced or modified

NS-B50027-4 produces omega-3 LC-PUFAs in seed oil, with a high level of DHA, a small amount of EPA and DPA. The oil fraction also contains a significant level of alpha-linolenic acid. Whereas alpha-linolenic acid can be derived from plants, the primary producers of EPA and DHA are mainly marine microalgae. EPA and DHA are concentrated in the food chain of marine fish, especially oily fish species. EPA and DHA are therefore often referred to as marine omega-3 fatty acids.

Seven genes involved in fatty acid biosynthesis were introduced to convert oleic acid into DHA in the seeds of oilseed rape NS-B5ØØ27-4. Oleic acid is converted to linoleic acid by desaturase Lackl-Δ12D, linoleic acid is desaturated to alpha-linolenic acid by Picpa-ω3D, alpha-linolenic acid to stearidonic acid by Micpu-Δ6D, stearidonic acid is converted to eicosatetraenoic acid by Pyrco-Δ6E, ETA to EPA by Pavsa-Δ5D, EPA to DPA by Pyrco-Δ5E and DPA to DHA by Pavsa-Δ4D (Figure 1).

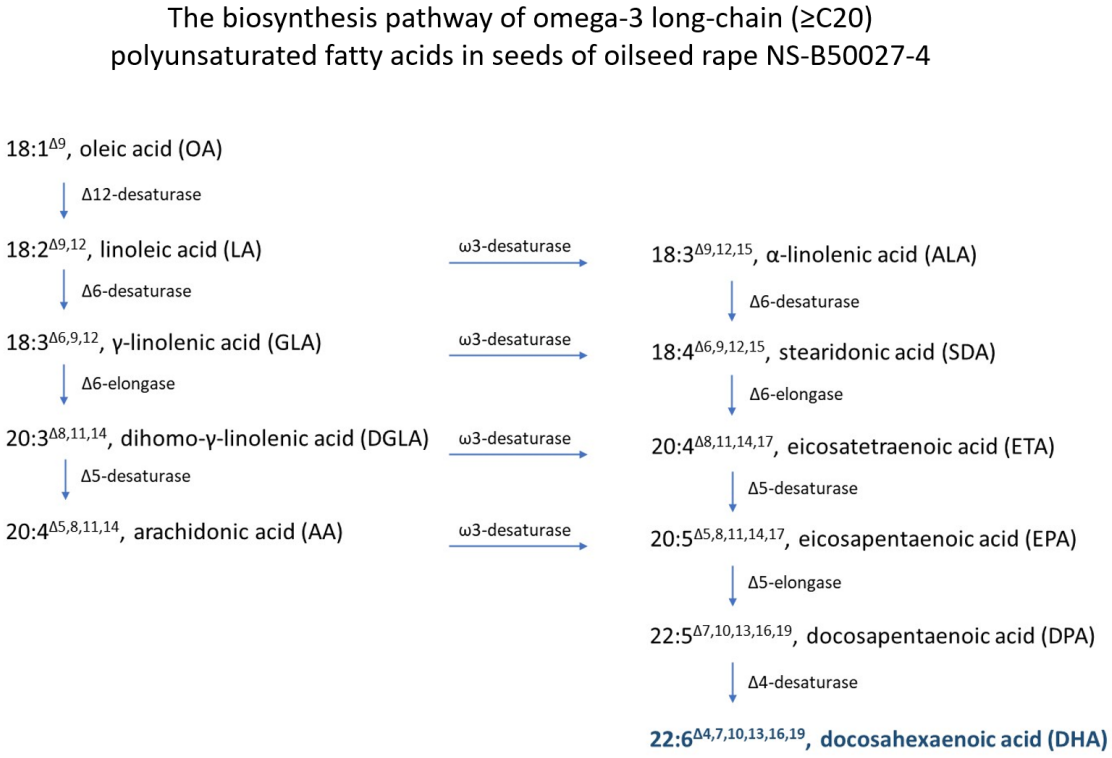

Figure 1. *Biosynthesis of fatty acids in the seeds of oilseed rape NS-B50027-4. The new enzymes expressed from the transgenes are indicated in the pathway. Adapted from Petrie J.R., et al. 2020.*

An eighth gene, *pat*, was introduced to encode the enzyme phosphinothricin N-acetyltransferase (PAT) which confers tolerance to glufosinate ammonium-based herbicides and was used as a selectable marker in the transformation process to develop oilseed rape NS-B5ØØ27-4.

3.2.2 Information on the sequences actually inserted

Oilseed rape NS-B5ØØ27-4 was characterised with vector-targeted sequencing, whole-genome sequencing (WGS) and PCR amplicon sequencing. The copy number of the inserted T-DNA was confirmed by Southern blot analysis.

The results from the sequencing analysis revealed that NS-B5ØØ27-4 had at least one set of eight genes from the binary vector pJP3416_GA7-ModB and no vector backbone sequence including the antibiotic resistance marker gene (*nptIII*). Southern blot analysis confirmed the sequencing results showing no vector backbone sequences in oilseed rape NS-B5ØØ27-4.

Furthermore, the sequencing data showed that oilseed rape NS-B5ØØ27-4 contained two T-DNA inserts, one on chromosome A05 and the other on chromosome A02. The sequence of each inserted T-DNA perfectly matched the reference of vector pJP3416_GA7-ModB, and no

amino acid variations were observed in the protein sequences of the eight genes compared to their references.

The A02 T-DNA insert has only four fatty acid synthesis gene cassettes, while the A05 T-DNA insert has a duplicated eight-gene set. The A02 T-DNA insert is 12,110 bp long, while the A05 T-DNA insert is 46,614 bp long. The integration of the T-DNA inserts into the oilseed rape NS-B5ØØ27-4 genome did not disrupt the expression of the eight genes.

The A02 T-DNA insert replaced 15 bp of *Brassica napus* genomic DNA sequence from the 3' UTR of a hypothetical protein *hpp* gene of unknown function. The *hpp* gene is located on chrUn_random of *Brassica napus* reference genome (Darmor) at position 118,589,903 – 118,591,677 and on chromosome A02 of *Brassica rapa* reference genome (Chiifu) at position 18,569,298 – 18,571,066.

The A05 T-DNA insert replaced a 20 bp of *Brassica napus* genomic DNA sequence from the second exon of a putative Pto-Interacting (*pti*) gene of unknown function. The *pti* gene is located on chromosome A05 of *B. napus* reference genome (Darmor) at position 17,267,746 – 17,270,700.

Both *hpp* and *pti* are uncharacterised genes from predicted sequences. Genome-wide analysis identified multiple sequences that have high sequence identity percentages to the *hpp* or the *pti* genes. The deletion of the *pti* gene did not have deleterious effects on oilseed rape NS-B5ØØ27-4 based on the data from the breeding program and field trial observations. No significantly negative effects on agronomic traits of oilseed rape NS-B5ØØ27-4 were observed from the T-DNA insertion and endogenous gene interruption.

Sub-cellular location(s) of insert(s):

The location of inserts in oilseed rape NS-B5ØØ27-4 were identified through vector-targeted sequencing and the identified sequences were compared using Basic Local Alignment Search Tool (BLAST) with sequences of reference genomes of *Brassica*. One insert resides on chromosome A02, and the other on chromosome A05. Their location on the nuclear genome on separate chromosomes was confirmed by their independent Mendelian segregation in a crossing experiment.

3.2.3 Open Reading Frames (ORFs) present within the insert and spanning the junction sites.

Bioinformatics analyses were used to evaluate the transgenic loci sequences of oilseed rape NS-B5ØØ27-4 to identify open reading frames (ORFs). Bioinformatic evaluations of the genetic sequences across the newly inserted T-DNA in each chromosome (A02 and A05) together with the insert-junctions were conducted to investigate possible similarities with known allergens using the AllergenOnline.org database (version 21), using full-length FASTA3, sliding window of 80 amino acids and exact word match for eight amino acid identity matches to known and putative allergens; and the NCBI Entrez Protein database

using BLASTp with keyword limit (allergen and allergy). Investigation of possible similarities with known toxins was performed using BLASTp with keyword search limits (toxic and toxin). No newly expressed ORFs that could raise any safety concern were identified.

The results from the bioinformatics analyses of the potential fusion protein amino acid sequences compared to known and putative allergens or toxins identified no significant sequence identity matches with any known allergens or toxins.

VKM concludes that the applicant has adhered to the recommendations of the EFSA guidance (EFSA, 2011a). The applicant has provided a sufficient description of the introduced traits and their mode of action, characteristics which have been introduced, and detailed information on organisation of inserted sequences, sub-cellular locations of the inserts, and bioinformatics analyses of open reading frames (ORFs) created within the insert and spanning the junction sites. Sufficient information about bioinformatic analyses performed to investigate possible similarities with known toxins or allergens using up-to-date databases were provided by the applicant.

3.2.4 Information on the expression of the inserted/modified sequence

Oilseed rape NS-B5ØØ27-4 was produced using *Agrobacterium tumefaciens* to integrate the *Lackl-Δ12D, Picpa-ω3D, Micpu-Δ6D, Pyrco-Δ6E, Pavsa-Δ5D, Pyrco-Δ5E* and *Pavsa-Δ4D* genes in the genome to produce DHA, and *pat* as a selectable marker gene.

The expression levels of the eight newly expressed proteins in oilseed rape NS-B5ØØ27-4 (treated with a conventional herbicide regime (CHR), treated with glufosinate plus a conventional herbicide regime (GHR)) were examined from samples obtained from field grown oilseed rape collected in the United States and Canada during the 2020 growing season.

A high sensitivity liquid chromatography-mass spectrometric multiple reaction monitoring (LC-MRM-MS) method was used to quantify the protein concentration levels of each of the expressed proteins in oilseed rape NS-B5ØØ27-4. Protein expression data related to mature seeds are considered the most relevant. In total, 298 test samples were analysed during the life cycle of NS-B5ØØ27-4 within different tissues.

NS-B5ØØ27-4 expresses eight novel proteins, Lackl-Δ12D, Picpa-ω3D, Pyrco-Δ6E, Pyrco Δ5E, Micpu-Δ6D, Pavsa-Δ5D, Pavsa-Δ4D and PAT. The protein expression levels of the seven DHA biosynthetic pathway enzymes were only measured in seeds since the genes encoding the proteins all have seed-specific promoters. Their expression is expected in seeds but absent in other tissues. PAT was measured in different tissues collected at different developmental stages, as expected from a constitutive promoter.

The seven DHA pathway proteins were only detected in developing and mature seeds of NS-B5ØØ27-4. The levels for all seven proteins were low, with Pyrco-Δ5E having the lowest and

Pavsa-Δ4D the highest expression. The *pat* gene regulated by a constitutive promoter was shown to be expressed in all examined parts of the modified plant.

While the ranges of expressed proteins are wide, comparison of mean values for NS-B5ØØ27-4 enzymes and the PAT protein of the CHR and GHR herbicide treatments in NS-B5ØØ27-4 were very similar. These results demonstrate that there is no effect of herbicide regimes on the concentration of the newly expressed proteins.

VKM concludes that the applicant has adhered to the recommendations of the EFSA guidance (EFSA, 2011a). The applicant has provided sufficient information demonstrating that the inserted/modified sequences resulted in intended changes at the protein level. Description of the used method, and data on the expression levels of the newly expressed proteins (the range and mean values for the produced proteins) were provided.

3.2.5 Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant

NS-B5ØØ27-4 was characterised with multiple sequencing approaches on plants from different breeding generations, and the sequencing results revealed that the transgenic loci in NS-B5ØØ27-4 are stably maintained across different breeding generations. Furthermore, the NS-B5ØØ27-4 genome was characterised by Southern blot analysis to confirm the stability of T-DNA inserts. The results showed that the T-DNA inserts were stable in NS-B5ØØ27-4 across multiple generations. The number and size of hybridised bands matched to the expected pattern based on the genome sequence, confirming the genome sequencing results.

Further, the phenotypic stability of the introduced traits in NS-B5ØØ27-4 was determined by means of lateral flow strip in several generations (20 plants from each generation were randomly selected). Samples were tested for glufosinate-tolerance via the PAT protein lateral flow strip test. The PAT protein was consistently detected in the tested generations and confirmed that the phenotype for the expressed trait in NS-B5ØØ27-4 is stable over multiple generations.

Mendelian inheritance was determined for each locus separately by genotype. F2 progeny plant tissue was screened with digital-PCR to estimate the locus copy number value based on specific genes (i.e., *pat*, *Micpu-Δ6D*) and markers (KASP assay; Kompetitive Allele-Specific PCR) specific to each locus T-DNA insert. The results from segregation analyses revealed that heritability and stability of the inserts in NS-B5ØØ27-4 were as expected, i.e., segregating in a Mendelian fashion.

VKM concludes that the applicant has adhered to the recommendations of the EFSAs guidance (EFSA, 2011a). The applicant has provided sufficient information demonstrating the genetic stability of the transgenic loci, the phenotypic stability, and inheritance patterns of the introduced traits.

3.3 Conclusions on the molecular characterisation

The provided scientific documentation regarding the molecular characterisation, i.e., description of sequences intended for insertion, actual inserted sequences, insertion sites, flanking sequence, new open reading frames etc., are adequate for risk assessment of oilseed rape NS-B5ØØ27-4 and in accordance with the EFSA guidance on risk assessment of genetically modified plants for use in food or feed.

VKM concludes that the molecular characterisation data on oilseed rape NS-B50027-4 indicate no increased risks compared to its conventional counterpart or commercial reference varieties.

4 Comparative assessment

EFSAs Guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011a) requires a comparative risk assessment of the GM plant and derived food and feed with appropriate comparators. The comparative assessment shall include compositional, agronomic as well as phenotypic characteristics, together with the molecular characterisation.

If significant differences in composition (e.g., nutrients), agronomic performance (e.g., flowering time) or other characteristics are found, these should be further investigated for biological relevance with respect to potential impact on human and animal health.

4.1.1 Production of material for the comparative assessment

The information on the production of the material for the comparative assessment was provided. High-quality of test materials was ensured by production of seed lots of oilseed rape NS-B5ØØ27-4 and the conventional counterpart (AV Jade) following best agronomic practices to ensure the identity, purity and health of the seed. The seed quality of the non-GM commercial reference varieties used in the field trials was comparable to commercialised varieties.

4.1.2 Criteria for selection of comparators

The EFSAs guidance defines requirements and recommendations for the selection and use of comparators. EFSA recommends using comparators that are either "the conventional counterpart" i.e., the non-GM isogenic variety (in the case of vegetatively propagated crops) or a genotype with a genetic background as close as possible to the GM plant (in the case of crops that are propagated sexually).

The applicant has adhered to the EFSA recommendations and included a conventional counterpart as comparator in addition to six non-GM commercial reference varieties.

4.1.3 Field trials: experimental design and statistical analysis

4.1.3.1 Experimental design

EFSAs Guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011a) requires applicants to adhere to the EFSA guidance on experimental design for the safety evaluation of GM plants provided in "Statistical considerations for the safety evaluation of GMOs" ((EFSA, 2010d; van der Voet et al., 2011)).

The applicant has adhered to the EFSA recommendations.

4.1.3.2 Statistical analysis

EFSAs guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011a) requires analysis of data in a clear format, using standardised scientific units. Adhering to the EFSA guidance on the statistical analysis for the safety evaluation of GM plants provided in "Statistical considerations for the safety evaluation of GMOs" (EFSA, 2010d) is recommended.

The applicant has adhered to the EFSA recommendations.

4.1.4 Compositional analysis

The EFSAs guidance requires the applicant to provide adequate comparative compositional analyses on a range of compounds. These include key-nutrients and anti-nutrients, amino acids, natural toxins and allergens or other metabolites characteristic for the plant species. Depending on the genetic modification and intended use of the plant, specific analyses may be required. E.g., in the case of oilseed rape NS-B5ØØ27-4, the intended changes in fatty acid composition must be demonstrated and compared to the comparator. Other significant (unintended) differences between the GM-plant and comparator should also be accounted for.

The applicant has adhered to the recommendations in the EFSA guidance. Apart from the intended changes in the fatty acid profile, and the expression of the new proteins, no biologically relevant differences are indicated in the comparative analyses.

4.1.5 Composition of oil fraction

The intended fatty acid modifications were demonstrated in the compositional analysis of oilseed rape NS-B5ØØ27-4, the conventional counterpart and reference varieties. It is shown that the crude fat levels of NS-B5ØØ27-4 (treated with conventional herbicide regime, CHR) and NS-B5ØØ27-4 (treated with glufosinate plus the conventional herbicide regime, GHR) were significantly different from the conventional counterpart and were not equivalent to the reference varieties tested. However, irrespective of herbicide treatments, means for fatty acids, except from C18:1 to C22:6, were within the range of the natural variation of reference varieties. Means for fatty acids C18:1 - C22:6 have changed as they are associated with the genetic modifications introduced. Therefore, the differences were considered not biologically relevant. The content of C18:0 (stearic) was not significantly different from the conventional counterpart but not equivalent to the non-GM commercial reference varieties. The content of fatty acids C24:0 (lignoceric), C22:0 (behenic), C20:1 (eicosenoic), C16:1 (palmitoleic) and C16:0 (palmitic) was significantly different from the conventional counterpart but were equivalent to the reference varieties. Mean levels of C18:3 ω3 (α−linolenic), C18:2ω6 (linoleic), C18:1 total, C18:1 ω9 (oleic) were significantly different from the conventional counterpart and were not equivalent to the reference varieties, as expected due to the genetic modifications introduced.

4.1.6 Processed oil to be used in fish feed

It is envisioned to use the oil derived from oilseed rape NS-B5ØØ27-4 in the production of feed for the aquaculture industry. The processing of oilseed rape grain into oil partitions removes all proteins to the meal fraction. The OECD Consensus document (OECD, 2011) recommends protein analysis in the meal fraction for animal feed, while protein analysis is not required for the oil fraction. Therefore, the applicant claims that the dietary intake of the newly expressed proteins in refined oil derived from NS-B5ØØ27-4 in aquaculture production will be negligible. Fish trials have shown that oil from NS-B5ØØ27-4 included in fish diets did not negatively affect results, i.e., the fish, compared to fish fed conventional feed (Hatlen et al., 2022; Ruyter et al., 2022; Ruyter et al., 2019). Also, the nutritional composition of meal from NS-B5ØØ27-4 reflects what was found in the analysis of whole grain.

4.1.7 Agronomic traits and GM phenotype

The applicant performed agronomic and phenotypic comparative assessments with the following selected endpoints: plant biology and morphology, agronomic performance, and common breeding parameters. These endpoints were measured throughout the growing season for each individual plot at each field trial on the complete set of entries, including the selected non-GM reference varieties. The comparative assessment showed that many continuous agronomic and phenotypic characteristics of NS-B50027-4 plants had no statistically significant differences compared to the characteristics of the non-GM conventional counterpart or were equivalent (or more likely equivalent than not) to those of the reference varieties. The comparative assessment of the agronomic and phenotypic characteristics indicates that NS-B5ØØ27-4 was comparable to the non-GM conventional counterpart and the commercial reference varieties in terms of the agronomic and phenotypic characteristics measured, and there were no unexpected or unintended effects due to the genetic modifications.

4.1.8 Effects of processing

According to the applicant report, refined oil from NS-B5ØØ27-4 seeds, is no different from conventional oilseed rape oils, except for the intended compositional fatty acid profile. The applicant has presented a detailed overview of the processing procedure which does not differ from production of conventional oilseed rape oils. To prevent thermal degradation of the longer-chain fatty acids, cooking temperatures are reduced during processing. The applicant presented results from the study confirming that the effects of processing on oil from NS-B5ØØ27-4 are not different from the effects on conventional oilseed rape oils. Other than the lowered cooking temperatures, no new production or manufacturing processes are envisaged for oil from oilseed rape NS-B5ØØ27-4.

4.2 Conclusions on the comparative assessment

Overall, the results of the comparative assessment (seed composition data and agronomic and phenotypic characteristics) presented by the applicant demonstrate that oilseed rape NS-B50027-4 is equivalent to its conventional counterpart and to the set of commercial reference varieties, except for the new introduced modified fatty acid profile and related pathway enzymes, as well as the PAT-enzyme. NS-B50027-4 oil is comparable to conventional oilseed rape oils, with the main considerable difference relating to the fatty acids (from C18:1 to C22:6) associated with the introduced genetic modifications.

5 Toxicological assessment

The purpose of the toxicological assessment is to demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health. It should also ensure that any unintended effect(s), revealed as significant differences compared to the conventional counterpart (comparator) variant in the molecular, compositional or phenotypic analyses, have no adverse effects on human and animal health.

5.1 Toxicological assessment of the newly expressed protein(s)

The knowledge available on the newly expressed protein(s) regarding the protein's source, function and if the protein previously has been used as food and feed for human/animals, decides the amount of toxicity testing required.

The applicant should provide information on:

a) molecular and biochemical characterisation of the newly expressed protein

b) up-to-date search for homology to known toxic proteins

c) stability of the protein under the relevant processing and storage conditions

d) resistance of the newly expressed protein to proteolytic enzymes (e.g., pepsin)

e) repeated dose toxicity studies using laboratory animals, unless reliable information demonstrating the safety can be provided

5.1.1 Molecular and biological characterization of the newly expressed proteins

The applicant should provide a molecular and biochemical characterization of the newly expressed protein(s), including the amino acid sequence, molecular weight, post-translational modifications, and a description of the function(s).

The newly expressed proteins in oilseed rape NS-B50027-4, producing Aquaterra® oil in the seeds, are the seven DHA pathway enzymes Lackl-Δ12D, Picpa–ω3D, Micpu-Δ6D, Pyrco-Δ6E, Pavsa-Δ5D, Pyrco-Δ5E and Pavsa-Δ4D for DHA and EPA synthesis and the enzyme PAT that confers herbicide tolerance.

The DHA pathway proteins are specifically expressed in the developing and mature seed. Pyrco-Δ6E was measured in immature seed materials, but not detected in mature seed. The PAT protein is present throughout the whole oilseed rape plant, while low amounts of Lackl-

Δ12D, Picpa–ω3D, Micpu-Δ6D, Pyrco-Δ6E, Pavsa-Δ5D, Pyrco-Δ5E and Pavsa-Δ4D was shown.

The studies of equivalency comparisons of newly expressed proteins and host-expressed intended protein should include: 1) size (SDS-PAGE), 2) immunogenicity (Western blot), 3) functional equivalence, 4) identical N-terminal sequence, 5) glycosylation.

The applicant showed that the DNA sequences inserted are translated to the intended new proteins of correct size. Immunogenicity by Western blot was not possible, since no antibodies are available to perform immunogenicity study. Further, the expression levels of the enzymes were too low to verify the biological function. Since it was not possible to purify enzymes from the plant, no data on glycosylation of these enzymes are available. However, the applicant performed a characterisation report identifying potential glycosylation sites.

The sources of the enzymes were described (derived from yeasts and microalgae) and not considered allergenic. A history of safe use in food and feed was also shown for the used sources.

VKM concludes that the applicant sufficiently has characterized the newly expressed proteins and no apparent modifications of the proteins were detected.

5.1.2 Bioinformatic search for homology to proteins known to cause adverse effects

The applicant should perform a search for homology to known toxic proteins and specify the database(s) and the methodology used to carry out the search.

The applicant compared the sequences of the newly expressed proteins to known toxins in the NCBI Protein database. In addition, a literature search for studies that might indicate possible risks of hazards from the sources and proteins was performed. Some short and modest identity matches to diverse proteins were found and most of the significant matches corresponded to fatty acid desaturase or acetyltransferase sequences, but not to any known toxic protein.

VKM concludes that homology between the newly expressed proteins to any known toxin was properly assessed with no toxin-like sequence identified.

5.1.3 Stability of the newly expressed proteins under relevant processing and storage conditions and the expected treatment of the food and feed

The applicant should assess whether or not the processing and/or preserving technologies applied are likely to modify the characteristics of the GM end-products differently than their comparator. The applicant should provide a detailed description of the processing

technologies used on the plant, paying special attention to the steps which may lead to significant changes in composition, both with respect to quality and quantity.

Oilseed rape seeds are subjected to different treatments during processing including heating to temperatures up to 80°C-105°C in various process steps. The applicant assessed the influences of temperature and pH changes on protein stability for Lackl-Δ12D, Picpa–ω3D, Micpu-Δ6D, Pavsa-Δ5D, Pyrco-Δ5E, Pavsa-Δ4D, and PAT. Protein stability was not tested for Pyrco-Δ6E. The reference standard received for Pyrco-Δ6E was not of sufficient purity for LC-MS/MS analysis, therefore no reportable Pyrco-Δ6E peptide results were obtained for any experiments performed in this study. The applicant showed that the evaluated proteins did not fully degrade under the different heat (up to 30 min at 90°C) and pH conditions tested (ranging from acidic to basic). However, even after cooling, it is unlikely that membrane proteins refold correctly and retain their enzymatic activity. Therefore, it is anticipated that the newly expressed transmembrane proteins in oilseed rape NS-B50027-4 will not be present in their native, folded state after processing and hence will have reduced or no activity. For enzymes, it can best be monitored using the enzyme activity, however *in vitro* assays to measure the enzyme activity of membrane proteins are rarely achieved and have not been possible to develop for the membrane proteins in oilseed rape NS-B50027-4.

VKM concludes that the proteins are only partly degraded by heat and low pH, but their enzymatic function might however be reduced. There are no good available assays to assess this. The oil fraction is, however, considered to be free of proteins after processing equivalent to conventional oilseed rape oils.

5.1.4 Resistance of the newly expressed proteins to proteolytic enzymes

Data concerning the resistance of the newly expressed protein to proteolytic enzymes (e.g., pepsin), should be performed by standardized *in vitro* tests. Stable breakdown products should be characterized and evaluated with regard to the potential risks associated with their biological activity.

If there is a possibility for synergistic or antagonistic interactions between two or more newly expressed proteins that may impact on safety, the applicant should perform additional studies with combined administration of these proteins.

The applicant showed that simulated gastric fluid (a 2-step pepsin and trypsin treatment) rapidly degraded the assessed proteins up to 93% during the 60 min experiment.

VKM concludes that the proteins are almost totally degraded by gastric enzymes.

5.1.5 Repeated-dose 28-day oral toxicity study with the newly expressed proteins in rodents

Repeated dose toxicity studies using laboratory animals should be provided, unless reliable information demonstrating the safety of the newly expressed protein (including its mode of action) can be provided, and it is demonstrated that the protein is not structurally and functionally related to proteins adversely affecting human or animal health. The repeated dose 28-day oral toxicity study in rodents with the newly expressed protein should be performed according to OECD guideline 407 (OECD, 2008). Depending on the outcome of the 28-day toxicity study, further targeted investigations may be required.

There were no scientific reasons to perform toxicology studies on the newly expressed proteins. The applicant did not perform a repeated dose 28-day oral toxicity test with the newly expressed proteins, since the enzymes are not expressed in high enough amounts and isolation of membrane proteins is not feasible. However, a 28-day toxicity study with NS-B50027-4 oil was performed in rats according to OECD guideline with no treatment related adverse effect identified (Murillo et al., 2021).

5.1.6 Toxicological assessment of new constituents other than proteins constituents, which may also require toxicological testing.

If there is a documented history of safe use and consumption as food and/or feed for the new constituents, then toxicological testing is not needed.

NS-B50027-4 was developed to contain the omega-3 LC-PUFAs, EPA, DPA and DHA. These fatty acids are not synthesised by conventional oilseed rape. There is a history of safe use of eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), found in seafood, including fatty fish or algal oils.

VKM concludes that no further toxicological studies were needed based on the previous assessments.

5.1.7 Design and performance of 90-day feeding study in rodents

If the composition of the food and/or feed derived from GM plant is substantially modified, or if there are any indications for the potential occurrence of unintended effects based on the preceding molecular, compositional or phenotypic analyses, not only new constituents but also the whole food and feed derived from the GM plant should be tested. In such case the testing program should include a 90-day toxicity study in rodents (EFSA, 2011b).

The applicant refers to two independent 90-day toxicity studies previously performed elsewhere on DHA rich algal or fish oils without any signs of treatment-related effects and claims that no further testing is needed. Nevertheless, even without indication of adverse effects, a 90-day feeding study with Aquaterra® oil, and NS-B50027-4 meal, in rats was

performed, as it is a mandatory requirement by Commission Implementing Regulation (EU) No 503/2013. The study design was based on the OECD Guideline 408 (1998), revised in 2018 (OECD, 2018). However, only one dose of each oil and meal was tested as no potential hazards were identified from preceding analyses.

The applicant showed that no toxicologically significant effects were seen on any of the clinical pathology parameters, ocular abnormalities, or changes in body weights, food consumption, clinical pathology parameters, or organ weights.

VKM concludes that a 90-day toxicity study is not scientifically justified based on the molecular and compositional assessment not indicating any interactions between the newly expressed protein and the plant's other protein synthesis. The applicant adhered to the guidelines and performed all required toxicity assessments. The results showed no toxicologically effects of the exposure.

5.1.8 Animal studies with respect to reproductive, developmental or chronic toxicity

Based on the above information and the weight of evidence, no more data are required to demonstrate that Aquaterra® oil and NS-B50027-4 meal is as safe as conventional oilseed rape oil and meal from a food and feed perspective.

5.1.9 Other animal studies to examine the safety and the characteristics of genetically modified food and feed

Based on the information provided above, there is no reason to expect any adverse or unintended effects from NS-B50027-4 oil and meal, and therefore additional animal studies are not scientifically justified.

5.1.10 Interpretation of relevance of animal studies

The results of both of the 28-day repeated dose oral gavage of oil and 90-day dietary whole food of meal and oil study showed no adverse effects and support the conclusion that Aquaterra® oil (and NS-B50027-4 meal) is as safe as conventional oilseed rape oil (and meal).

5.1.11 Allergenicity

Potential allergenicity should be assessed for the newly expressed proteins on a case-by-case approach or weight-of-evidence approach.

Typically, the weight of evidence evaluates:

1) the allergenic potential of the source of the gene and the host plant

2) in silico comparisons of the amino acid sequence of the newly introduced proteins against protein and allergen databases
3) the digestibility of the proteins using pepsin and/or trypsin enzymes in a standard protocol
4) the relative abundance of the introduced proteins compared to total protein.

A search for sequence homologies and/or structural similarities between the expressed protein and known allergens should be performed to identify potential IgE cross-reactivity between the newly expressed protein and known allergens. The alignment-based criterion involving 35 % sequence identity to a known allergen over a window of at least 80 amino acids is considered a minimal requirement (EFSA, 2010c).

All the newly expressed proteins were derived from sources that are not known to be allergenic, and homology searches in the AllergenOnline.org database did not match with known allergens. Nor did the literature search identify any publications showing that any of the donor organisms would induce human allergy.

VKM concludes that the applicant used the correct tools for the allergenicity assessment, and that no biologically relevant sequence-identity-match to known allergens were identified.

5.1.12 Assessment of adjuvanticity

Adjuvants are substances that, when co-administered with an antigen increase the immune response to the antigen and therefore might also increase the allergic response. In cases when the newly expressed protein is known to have structural similarity to strong adjuvants or the structure may indicate possible adjuvant activity, the possible role of the protein as adjuvant should be considered.

The applicant showed that the newly expressed proteins did not share any sequence or structural similarity to known adjuvants such as certain lectins and agglutinins.

VKM concludes that all requirements of allergenicity and adjuvanticity testing were fulfilled and no allergenic risk was reported.

5.2 Conclusions on the toxicological assessment

Overall, the results of the toxicological assessment (acute and sub chronic toxicity, sequence homology to known toxins or allergens, allergenic potential, stability to digestive enzymes or heat and structural similarity to known adjuvants) presented by the applicant demonstrate that there is no toxicological or allergenic risk linked to oilseed rape NS-B50027-4.

6 Exposure assessment

The refined oil, Aquaterra®, will be used as an alternative source of long chain marine omega-3 fatty acids in fish feed.

Oilseed rape, and fish oils rich in omega-3 fatty acids are widely used today in animal feed for cattle, swine, poultry, and many aquaculture species. Aquaterra® will be primarily offered to the aquaculture market as an alternative ingredient to fish oils in aquaculture diets but can also be utilised for other livestock feeds. NS-B50027-4 oil is already approved for fish feed in Australia, New Zealand, and Canada, marketed as Aquaterra®. The oil has also been developed for human nutrition, marketed as Nutriterra®.

Human exposure

Proteins may be found in unrefined oilseed rape oil, but not in refined oil (Martín-Hernández et al., 2008). The newly expressed proteins, Lackl-Δ12D, Picpa–ω3D, Micpu-Δ6D, Pyrco-Δ6E, Pavsa-Δ5D, Pyrco-Δ5E, Pavsa-Δ4D for the production of EPA, DPA and DHA, and PAT, are reported not to be present in the final refined Aquaterra® oil. The new constituents in Aquaterra® are EPA, DPA and DHA at a level of about 10% combined. Oils with these fatty acids are already consumed from fish oils, and the substitution with Aquaterra® is therefore not expected to influence the intake.

Occupational exposure to Aquaterra® in relation to fish feed and the production thereof is not considered to be different from exposure to other types of oilseed rape oils used in feeds.

7 Conclusions on the risk assessment of genetically modified oilseed rape NS-B5ØØ27-4 for food and feed uses in the EU

VKM has assessed the scientific documentation provided in application EFSA-GMO-NL-2019-160, relevant for the risk assessment of Aquaterra® for use as an ingredient in fish feed in Norway.

VKM concludes that the molecular characterisation, i.e., description of sequences intended for insertion, actual inserted sequences, insertion sites, flanking sequence, new open reading frames etc., are adequate for risk assessment and in accordance with the EFSA guidance. The molecular characterisation indicates no increased risks associated with oilseed rape NS-B50027-4 compared to its conventional counterpart or commercial reference varieties.

Likewise, results of the comparative assessment (compositional, agronomic and phenotypic analyses) presented by the applicant demonstrate that oilseed rape NS-B50027-4 is equivalent to its conventional counterpart and to the set of commercial reference varieties, except for the intended changes in fatty acids and associated new enzymes/proteins. Oil from NS-B50027-4 is equivalent to conventional oilseed rape oils, with the main considerable differences relating to the changes in fatty acids.

The results of the toxicological assessment (e.g., acute and sub chronic toxicity, homology to known toxins or allergens of inserted sequences, allergenic potential of new proteins or other constituents, stability to digestive enzymes or heat and structural similarity to known adjuvants) presented by the applicant demonstrate that there are no increased toxicological or allergenic risks linked to the new proteins/enzymes, or the plant itself compared to conventional oilseed rape varieties.

Based on this VKM concludes that derived oil from oilseed rape NS-B50027-4 indicates no increased risks to human or animal health compared to conventional oilseed rape oils.

Data gaps and uncertainties

VKM has not identified data gaps or uncertainties relevant to the risk assessment of Aquaterra® as ingredient in fish feed.

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Appendix 1

Risikovurdering av rapsoljen Aquaterra® til bruk som ingrediens i fiskefôr

Utvidet norsk sammendrag

Basert på risikovurderingen **«Risk assessment of Aquaterra® oil for its intended use as ingredient in fish feed»** fra Vitenskapskomiteen for mat og miljø (VKM Report 2023: 8).

For utfyllende informasjon vises det til hovedrapporten: https://vkm.no/

Bakgrunn for risikovurderingen

Vitenskapskomiteen for mat og miljø (VKM) har på oppdrag fra Mattilsynet, bestillingsdato 3.10.2022, vurdert en søknad om godkjenning av prosessert genmodifisert rapsolje, Aquaterra®, fra genmodifisert raps NS-B50027-4, utelukkende til import og bruk som ingrediens i fiskefôr i Norge.

Raps NS-B50027-4 produserer langkjedede (≥C20) flerumettede omega-3-fettsyrer (omega-3 LC-PUFA) i frøene, med et høyt nivå av dokosaheksaensyre (DHA) og små mengder eikosapentaensyre (EPA) og dokosapentaensyre (DPA). Aquaterra® inneholder også et betydelig nivå av alfa-linolensyre (ALA). ALA kan utvinnes fra planter, mens de primære produsentene av EPA og DHA hovedsakelig er marine mikroalger. EPA og DHA akkumuleres oppover i næringskjeden, spesielt i fet fisk i havet, og blir ofte referert til som marine omega-3 fettsyrer. EPA og DHA er essensielle fettsyrer for fisk og dermed viktige ingredienser i fiskefôr. Raps NS-B50027-4 ble utviklet som et plantebasert alternativ til marine fettsyrer, hovedsakelig DHA.

Raps NS-B50027-4 er genmodifisert til å uttrykke syv transgener avledet fra gjær og marine mikroalger. Transgenene koder for nødvendige enzymer i biosyntesen av de marine omega-3 fettsyrene. Et åttende transgen, *pat*, ble satt inn som seleksjonsmarkør under utviklingen av rapsen. *Pat*-genet koder for enzymet fosphinothricin N-acetyltransferase (PAT), som gir rapsen økt toleranse for ugressmidler basert på glufosinat-ammonium. I likhet med konvensjonelle prosesserte rapsoljer vil eventuelle rester av proteiner, inkludert de introduserte enzymene, være ubetydelig i oljen Aquaterra®.

Mattilsynets mandat til VKM

1. VKM skal vurdere mulige negative helseeffekter hos fisk fôret med den genmodifiserte rapsoljen Aquaterra®. Risikovurderingen skal ha særskilt fokus på oljen til bruk i fiskefôr til arter i laksefamilien (*Salmonidae*), inkludert atlantisk laks (*Salmo salar*).

2. Dersom VKM anser det nødvendig, skal det vurderes utilsiktede skadelige miljøkonsekvenser ved bruk av fiskefôr med innhold av Aquaterra® rapsolje. Hvis VKM ikke finner at dette er relevant, skal det begrunnes.

3. Basert på risikovurderingen ber Mattilsynet VKM vurdere om det er behov for en overvåkingsplan for å avdekke framtidige utilsiktede effekter på helse og miljø.

Om risikovurderingen

VKMs risikovurdering av Aquaterra® ble utført i samsvar med veiledningen fra Den europeiske myndighet for næringsmiddeltrygghet (EFSA) for risikovurdering av genmodifiserte planter til bruk i mat og fôr. Risikovurderingen er i all hovedsak basert på dokumentasjon fra søknaden 'EFSA-GMO-NL-2019-160', om godkjenning av raps NS-B50027-4 til aktuelle bruksområder innen mat- og fôr på linje med konvensjonelle rapssorter i EU,

med unntak av dyrking. Fagområdene som dekkes av risikovurderingen inkluderer molekylær karakterisering, komparative analyser av ernæringsmessig innhold, toksikologiske analyser og analyser av allergent potensiale av raps NS-B50027-4 og prosesserte produkter.

Den vitenskapelige dokumentasjonen i søknaden EFSA-GMO-NL-2019-160 har tidligere blitt vurdert av VKMs faggruppe for GMO i forbindelse med EFSAs vitenskapelige høring i 2022, som del av EFSAs (pågående) risikovurdering av raps NS-B50027-4.

Supplerende vitenskapelig litteratur benyttet i risikovurderingen av Aquaterra® ble skaffet via frie litteratursøk utført av prosjektmedlemmer, i tillegg til et systematisk litteratursøk utført av Folkehelseinstituttet - biblioteket.

Gjennomgang av relevant søknadsdokumentasjon, vitenskapelig litteratur og sammenstilling av risikovurderingen, ble utført av en oppnevnt gruppe bestående av seks av VKMs komitemedlemmer koordinert av prosjektleder fra VKMs sekretariat. To eksterne fagfeller gjennomgikk og ga innspill til rapportutkastet før det ble godkjent av VKMs faggruppe for genmodifiserte organismer supplert av et medlem fra faggruppen for fôr.

Om genmodifiseringen i raps NS-B50027-4

Rapsen NS-B50027-4 er utviklet ved transformasjon av frøblad (kimblad) fra spirende frø av rapslinjen AV Jade, ved bruk av jordbakterien *Agrobacterium tumefaciens. A. tumefaciens* er et plantepatogen som normalt forårsaker svulster/galler i planter ved å overføre spesifikke gener til plantevevet den smitter. Bakterien er mye brukt innen genmodifisering ved at man bruker dens naturlige mekanisme for smitte (overføring av gener), men bytter ut genene bakterien vanligvis overfører med ønskede gener. I dette tilfellet gener som koder for syv enzymer som inngår i biosyntesen av omega-3-fettsyrer i frøene til rapsen (figur 1), og et åttende markørgen for toleranse mot ugressmiddelet glufosinat-ammonium.

De åtte genene som koder for de syv enzymene som inngår i biosyntesen av fettsyrer, pluss enzymet for ugressmiddeltoleranse, er hentet fra følgende to gjærsopper, tre marine mikroalger og én bakterie:

1. Enzymet Δ12-desaturase (fra genet *Lackl-Δ12D*) er opprinnelig fra gjærsoppen *Lachancea kluyveri,* som blant annet brukes til å lage ulike oster, f.eks. Emmental og Roquefort.

2. Enzymet ω3-/Δ15-desaturase (fra genet *Picpa-ω3D*) opprinnelig fra gjærsoppen *Pichia pastoris*, som brukes blant annet til dyrefôr, samt til produksjon av ulike proteiner og enzymer innen matproduksjon.

3. Enzymet Δ6-desaturase (fra genet *Micpu-Δ6D*) fra den marine mikroalgen *Micromonas pusilla. M. pusilla* er en viktig næringskilde for mange organismer i havet, og brukes som fôr til østers-larver.

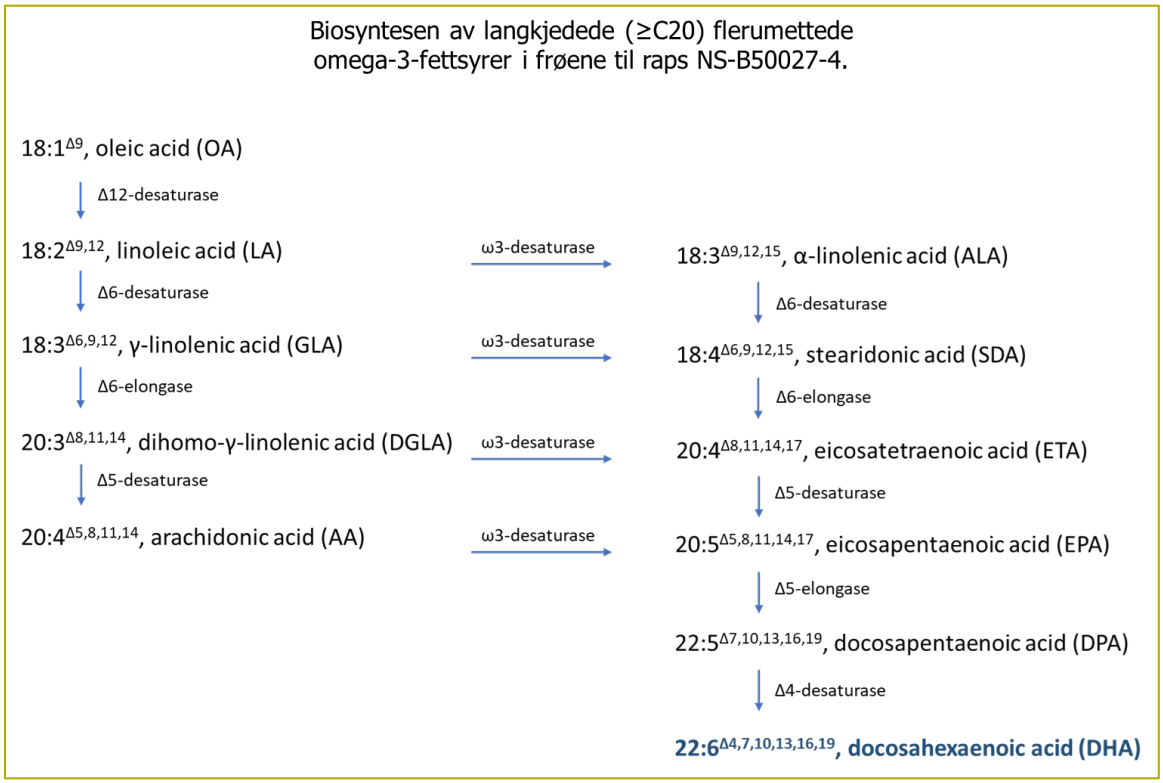
4. Enzymet Δ6-elongase (fra genet *Pyrco-Δ6E*) fra mikroalgen *Pyramimonas cordata. P. cordata* spiller en viktig rolle i den marine næringskjeden, og har blitt brukt til isolering av fytosteroler.

5. Enzymet Δ5-desaturase (fra genet *Pavsa-Δ5D*) fra mikroalgen *Pavlova salina. Pavlova salina* brukes også som fôr til østers-larver.

6. Enzymet Δ5-elongase (fra genet *Pyrco-Δ5E),* også fra mikroalgen *Pyramimonas cordata.*

7. Enzymet Δ4-desaturase (fra genet *Pavsa-Δ4D),* også fra mikroalgen *Pavlova salina.*

8. Enzymet fosphinothricin N-acetyltransferase (fra genet *pat*), som gir plantene økt toleranse for ugressmiddelet glufosinat-ammonium, er hentet fra bakterien *Streptomyces viridochromogenes.*



Figur 1: De syv nye enzymene som inngår i dannelsen av fettsyrer, uttrykt av transgenene i raps NS-B50027-4, er indikert i synteseveien. Adaptert fra Petrie J.R., et al. 2020.

Konklusjoner

VKMs konklusjoner som gjelder raps NS-B50027-4

VKM konkluderer at den vitenskapelige dokumentasjonen vedlagt i søknaden EFSA-GMO-NL-2019-160, oppfyller kriteriene i EFSAs veiledning og er tilstrekkelig for risikovurderingen av raps NS-B50027-4.

VKMs konklusjon er at den molekylære karakteriseringen, de komparative, ernæringsmessige og toksikologiske analysene, og vurderingen av allergent potensiale av raps NS-B50027-4, ikke indikerer økt helserisiko for dyr eller mennesker sammenliknet med rapsens konvensjonelle motpart (komparator) eller kommersielle referansesorter.

VKMs konklusjoner som gjelder rapsoljen Aquaterra®

Basert på VKMs vurdering av den vitenskapelig dokumentasjon som gjelder raps NS-B50027-4, inkludert analyser av selve oljen Aquaterra® og fôringsstudier på b.la. fisk, konkluderer VKM at prosessert olje fra raps NS-B50027-4 tilsvarer konvensjonelle rapsoljer, med unntak av de tilsiktede endringene i fettsyrer.

VKMs konklusjoner til mandatet fra Mattilsynet

1. **Vurdering av mulige negative helseeffekter hos fisk fôret med Aquaterra® som ingrediens i fiskefôr, med særskilt fokus på fôr til arter i laksefamilien (*Salmonidae*)**

Basert på den vitenskapelige dokumentasjonen vedlagt i søknaden, og vitenskapelig litteratur, konkluderer VKM at det ikke er en økt helserisiko for fisk gitt fôr med Aquaterra® sammenliknet med konvensjonelt fôr med oljer fra andre kilder.

2. **Vurdering av utilsiktede skadelige miljøkonsekvenser ved bruk av fiskefôr med innhold av Aquaterra®. Hvis VKM ikke finner at dette er relevant, skal det begrunnes.**

Den vitenskapelige dokumentasjonen vedlagt i søknaden viser at den prosesserte oljen Aquaterra® tilsvarer konvensjonelle rapsoljer med unntak av de marine omega-3-fettsyrene, som allerede brukes i fiskefôr. VKM konkluderer derfor at det ikke er indikasjoner på en økt miljørisiko ved bruk av Aquaterra® i fiskefôr sammenliknet med konvensjonelt fôr med oljer fra andre kilder.

3. **Vurdere om det er behov for en overvåkingsplan for å avdekke framtidige utilsiktede effekter på helse og miljø.**

Ettersom Aquaterra® tilsvarer konvensjonelle rapsoljer med unntak av de marine omega-3-fettsyrene, som allerede brukes i fiskefôr, konkluderer VKM at det ikke er noe større behov for helse- eller miljøovervåking av fôr som inneholder Aquaterra® enn for konvensjonelt fiskefôr.

Appendix 2

Aquaterra® in fish feed

Contact:	Ville Erling Sipinen, VKM
Search made by:	Trude Anine Muggerud, NIPH
Internal Referee:	Nataliya Byelyey, NIPH
Removal of duplicates:	Result before removal: 125
	Result after removal: 79

Question to be answered by the literature search			
«Is there a potential health risk to farmed salmon or other salmonids in aquaculture with the use of "Aquaterra®" (a genetically modified oilseed rape/canola oil) in fish feed, provided the feed is nutritionally balanced and equal to other feeds where the "marine" fatty acids are derived from other sources?"			
Question in PICO-format			
Population	**Intervention**	**Comparison**	**Outcome**
	[Aquaterra®, Aquaterra® in fish feed, genetically modified canola oil, genetically modified DHA-canola oil, genetically modified DHA-rapeseed oil, genetically modified DHA-oilseed rape oil, genetically modified DHA-rape oil, genetically modified oilseed rape NS-B5ØØ27-4 oil, NS-B5ØØ27-4 oil, genetically modified plant NS-B5ØØ27-4 oil, GMP NS-B5ØØ27-4 oil, DHA Canola Line NS-B50027-4]		

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to 2023>
Result: 14

1	(Aquaterra or ("genetically modified DHA" adj ("canola oil?" or canolaoil? or "rapeseed oil?" or rapeseedoil? or "oilseed rape oil?" or "rape oil?" or rapeoil?)) or "DHA canola" or "DHA rapeseed" or "DHA rape" or "genetically modified canola oil?" or "genetically modified canolaoil?" or NS-B50027-4).tw,kf.	14

Database: Embase <1974 to 2023>
Result: 19

1	(Aquaterra or ("genetically modified DHA" adj ("canola oil?" or canolaoil? or "rapeseed oil?" or rapeseedoil? or "oilseed rape oil?" or "rape oil?" or rapeoil?)) or "DHA canola" or "DHA rapeseed" or "DHA rape" or "genetically modified canola oil?" or "genetically modified canolaoil?" or NS-B50027-4).tw,kf.	19

Database: Web of Science
Result: 32

#1	TS=("Aquaterra" OR ("genetically modified DHA" NEAR/0 ("canola oil$" OR "canolaoil$" OR "rapeseed oil$" OR "rapeseedoil$" OR "oilseed rape oil$" OR "rape oil$" OR "rapeoil$")) OR "DHA canola" OR "DHA rapeseed" OR "DHA rape" OR "genetically modified canola oil$" OR "genetically modified canolaoil$" OR "NS-B50027-4")	**32**

Database: CAB Abstracts <1973 to 2023>

Result: 60

1	(Aquaterra or ("genetically modified DHA" adj ("canola oil?" or canolaoil? or "rapeseed oil?" or rapeseedoil? or "oilseed rape oil?" or "rape oil?" or rapeoil?)) or "DHA canola" or "DHA rapeseed" or "DHA rape" or "genetically modified canola oil?" or "genetically modified canolaoil?" or NS-B50027-4).ti,ab.	60