



Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Preliminary environmental risk assessment of genetically modified oilseed rape MON 88302 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/BE/2011/101)

Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety

Scientific comments submitted to the EFSA GMO Extranet

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

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The Norwegian Veterinary Institute (NVI) has assessed the molecular characterization of oilseed rape MON 88302 in accordance with EFSA's Guidance document for risk assessment of food and feed from genetically modified plants (EFSA 2011a). VKM acknowledge NVI for their valuable work on this opinion.

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Summary

The environmental risk assessment of the herbicide tolerant genetically modified oilseed rape MON 88302 (Reference EFSA/GMO/BE/2011/101) has been performed by the Panel on Genetically Modified Organisms (GMO) of the Norwegian Scientific Committee for Food Safety (VKM). VKM has been requested by the Norwegian Directorate for Nature Management and the Norwegian Food Safety Authority to issue a preliminary scientific opinion on the safety of the genetically modified oilseed rape MON 88302 (Unique identifier MON-88Ø2-9) for food and feed uses, import and processing, and submit relevant scientific comments or questions to EFSA on the application EFSA/GMOBE/2011/101.

The environmental risk assessment of the MON 88302 is based on information provided by the applicant in the application EFSA/GMO/BE/2011/101, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered peer-reviewed scientific literature as relevant.

The VKM GMO Panel has evaluated MON 88302 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2006, 2011a), the environmental risk assessment of GM plants (EFSA 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2006, 2011c).

The scientific risk assessment of oilseed rape MON 88302 include molecular characterisation of the inserted DNA and expression of target proteins, comparative assessment of agronomic and phenotypic characteristics, unintended effects on plant fitness, potential for horizontal and vertical gene transfer, and evaluations of the post-market environmental plan.

In line with its mandate, VKM emphasized that assessments of sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act, shall not be carried out by the Panel on Genetically Modified Organisms. The GMO Panel has therefore not considered possible health and environmental effects of cultivation and processing of oilseed rape MON 88302 outside the EU/EEA area.

The genetically modified oilseed rape MON 88302 was developed to provide tolerance to the herbical active substance glyphosate by the introduction of a gene coding for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium tumefaciens*, strain CP4 (CP4 EPSPS). Glyphosate is a non-selective herbicide and is normally phytotoxic to a broad range of plants. Its mode of action occurs by binding to and inactivating the EPSPS protein, which is a key enzyme in the shikimate pathway that leads to the biosynthesis of the aromatic amino acids tyrosine, tryptophan and phenylalanine. The disruption of this pathway and the resulting inability to produce key amino acids prevents growth and ultimately leads to plant death.

Molecular characterisation

The VKM Panel on Genetically Modified Organisms find the conclusion that no major section of the T-DNA plasmid backbone is inserted in MON88302 oilseed rape justified. We also find it justified that there is only one major T-DNA insert in MON88302.

Comparative assessment

Based on results from comparative analyses of data from field trials located at representative sites and environments in the USA, Canada and Chile, it is concluded that oilseed rape MON 88302 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of the herbicide tolerance conferred by the CP4 EPSPS protein. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON 88302 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glyphosate herbicide do not alter the phenotypic and agronomic characteristics of MON 88302 compared to conventional oilseed rape.

Evaluations of environmental interactions between genetically modified oilseed rape MON 88302 and the biotic and abiotic environment, and studies of seed dormancy, seed germination, pollen morphology and viability indicates no unintended effects of the introduced trait on these characteristics in MON 88302 oilseed rape.

Environmental risk

Considering the scope of the application EFSA/GMO/BE/2011/101, excluding cultivation purposes, the environmental risk assessment is limited to exposure through accidental spillage of viable seeds of MON 88302 into the environment during transportation, storage, handling, processing and use of derived products.

Oilseed rape is mainly a self-pollinating species, but has entomophilous flowers capable of both self- and cross-pollinating. Normally the level of outcrossing is about 30 %, but outcrossing frequencies up to 55 % are reported.

Several plant species related to oilseed rape that are either cultivated, occurs as weeds of cultivated and disturbed lands, or grow outside cultivation areas to which gene introgression from oilseed rape could be of concern. These are found both in the *Brassica* species complex and in related genera. A series of controlled crosses between oilseed rape and related taxa have been reported in the scientific literature. Because of a mismatch in the chromosome numbers most hybrids have a severely reduced fertility. Exceptions are hybrids obtained from crosses between oilseed rape and wild turnip (*B. rapa* ssp. *campestris*) and to a lesser extent, mustard greens (*B. juncea*), where spontaneously hybridising and transgene introgression under field conditions have been confirmed. Wild turnip is native to Norway and a common weed in arable lowlands.

There is no evidence that the herbicide tolerant trait results in enhanced fitness, persistence or invasiveness of oilseed rape MON 88302, or hybridizing wild relatives, compared to conventional oilseed rape varieties, unless the plants are exposed to glyphosate-containing herbicides.

However, accidental spillage and loss of viable seeds of MON 88302 during transport, storage, handling in the environment and processing into derived products is likely to take place over time, and the establishment of small populations of oilseed rape MON 88302 on locations where glyphosate is frequently applied to control weeds e.g. on railway tracks, cannot be excluded. Feral oilseed rape MON 88302 arising from spilled seed could theoretically pollinate conventional crop plants if the escaped populations are immediately adjacent to field crops, and shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops. However, both the occurrence of feral oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape populations to wild populations are likely to be low in an import scenario. Apart from the glyphosate tolerance trait, the resulting progeny will not possess a higher fitness and will not be different from progeny arising from cross-fertilisation with conventional oilseed rape varieties.

The VKM GMO Panel concludes that this route of gene flow would not introduce significant numbers of transgenic plants into agricultural areas or result in any environmental consequences in Norway.

The environmental risk assessment will be completed and finalized by the VKM Panel on Genetically Modified Organisms when requested additional information from the applicant is available.

Keywords

Oilseed rape, *Brassica napus* ssp. *oleifera* (DC.) Metzg., genetically modified oilseed rape MON 88302, EFSA/GMO/BE/2011/101, herbicide tolerance, glyphosate, CP4 EPSPS protein, environmental risk, Regulation (EC) No 1829/2003, Directive 2001/18/EC

Norsk sammendrag

Miljøriskovurderingen av den genmodifiserte oljerapsen MON 88302 (EFSA/GMO/BE/2011/101) fra Monsanto Company er utført av Faggruppen for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet (VKM). VKM er bedt av Mattilsynet og Direktoratet for naturforvaltning om å vurdere helse- og miljørisiko ved en eventuell godkjenning av rapslinjen MON 88302 (unik kode MON-88Ø2-9) til import, prosessering og til bruk i næringsmidler og fôrvarer, samt gi relevante kommentarer og spørsmål til EFSA i forbindelse med den offentlige høringen av søknaden. Søknaden gjelder ikke dyrking.

Den foreløpige risikovurderingen av den genmodifiserte rapslinjen er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSA nettside EFSA GMO Extranet.

Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med miljø- og helsekravene i matloven og genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i EU-forordning 1829/2003/EF, utsettingsdirektiv 2001/18/EF (vedlegg 2,3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSA's retningslinjer for risikovurdering av genmodifiserte planter og avledede næringsmidler (EFSA 2006, 2010, 2011a) og Organisation for Economic Co-operation and Development (OECD) konsensusdokument for raps (OECD 2001, 2011) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsprosess, vektor, transgene konstrukt, komparative analyser av agronomiske og fenotypiske egenskaper, potensielle for ikke tilsiktede effekter på fitness, horisontal og vertikal genoverføring og søkers overvåkingsplan vurdert.

Det presiseres at VKMs mandat ikke omfatter vurderinger av etikk, bærekraft og samfunnsnytte, i henhold til kravene i den norske genteknologiloven og dens konsekvensutredningsforskrift. Disse aspektene blir derfor ikke vurdert av VKMs faggruppe for genmodifiserte organismer.

Rapslinjen MON 88302 er framkommet ved *Agrobacterium*-mediert transformasjon av hypokotyl (frøstengel) fra frøplanter fra den kommersielle vårrapssorten «Ebony». Den innsatte genkonstruksjonen i MON 88302 inneholder det bakterielle genet *cp4-epsps* fra *Agrobacterium tumefaciens*, linje CP4. *Cp4-epsps*-genet koder for enzymet 5-enolpyruvylsikimat-3-fosfatsyntetase (CP4 EPSPS), som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsikimat-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. N-fosfonometylglisin (glyfosat) er et systemisk, ikke selektivt herbicid som hemmer EPSPS-enzymet og som blokkerer biosyntesen av aromatiske aminosyrer i planter. I motsetning til plantens eget EPSPS-enzym er det bakterielle enzymet også aktivt ved nærvær av N-fosfonometylglisin. De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoff glyfosat sammenlignet med konkurrerende ugras.

Oljerapslinjen MON 88302 inneholder ingen markørgener for antibiotikaresistens.

Molekylær karakterisering

Den transgene rapslinjen MON 88302 har fått tilført genet *cp4 epsps*. I henhold til søkers informasjon vedrørende integreringsplass og flankesekvenser til det integrerte transgenet, samt analyser v.h.a. Southern blot og sekvensering er det grunn til å tro at transgenet sitter i et lokus i genomet. Det konkluderes med at nedarvingen av *cp4 epsps*-genet i rapslinjen MON 88302 følger mønsteret for mendelsk nedarving av et enkelt, dominant lokus, og at fusjonsproteiner ikke uttrykkes i MON 88302.

Faggruppen vurderer karakteriseringen av det rekombinante innskuddet i rapslinjen MON 88302, og de fysiske, kjemiske og funksjonelle karakteriseringen av proteinene til å være tilfredsstillende.

Faggruppen har ikke identifisert noen risiko knyttet til det som framkommer av den molekylærbiologiske karakteriseringen av de rekombinante innskuddene i rapslinjen.

Komparative analyser

Feltforsøkene som ligger til grunn for søkers komparative analyser er i tråd med EFSA's retningslinjer for risikovurdering av genmodifiserte planter og avledete mat- og fôrvarer (EFSA 2011a). Feltforsøk over en vekstsesong i USA, Canada og Chile viser små eller ingen signifikante forskjeller mellom den transgene oljerapslinjen MON 88302 (usprøytet og sprøytet med tiltenkt herbicid) og umodifisert, nær-isogen kontroll med hensyn på fenotypiske og agronomiske karakterer.

Miljørisiko

Søknad EFSA/GMO/BE/2011/101, omfatter godkjenning av genmodifisert oljeraps MON88302 til import, prosessering og til bruk i næringsmidler og fôrvarer. Miljørisikovurderingen av MON88302 er derfor knyttet til mulige effekter av utilsiktet frøspredning i forbindelse med transport, lagring og prosessering til mat og fôr.

Oljeraps er hovedsakelig en selvbestøvende art. Frekvensen av krysspollineringer er normalt om lag 30 %, men opp til 55 % utkryssing er registrert hos enkelte sorter. Rapspollen har både insekt- og vindspredning, og pollenet kan under gitte omstendigheter spres over store avstander. Induksjon av sekundær frøkvile og etablering av persistente frøbanker i jord gjør at rapsfrø kan være en kilde til uønsket genflyt over lengre tidsrom. Oljeraps har flere beslektede arter som enten dyrkes, opptrer som ugrasarter eller er viltvoksende utenfor dyrking i Norge. Dette gjelder både arter i *Brassica*-komplekset og andre arter i nærstående slekter. Genutveksling mellom dyrket rybs og raps skjer spontant. Det er også vist at oljeraps kan danne spontane hybrider med åkerkål (*B. rapa* ssp. *campestris*), et vanlig åkerugras i hele Sør-Norge. Transgener kan overføres til åkerkål ved tilbakekryssing i løpet av to generasjoner, en forutsetning for stabil integrering av transgener. Det er også rapport om spontan hybridisering i felt med sareptasennep (*B. juncea*), men hybridiseringsfrekvensene er svært lave og utbredelsen av arten er marginal i Norge.

Det er ingen indikasjoner på økt risiko for spredning, overlevelse og etablering av rasplinen MON88302 som naturaliserte populasjoner utenfor dyrkingsområder eller for utvikling av ugraspopulasjoner sammenlignet med ikke-transgen raps. Herbicidtoleranse er selektivt nøytralt i naturlige habitater, og kan bare betraktes å ha økt fitness hvor og når glyfosatholdige herbicider anvendes.

Transport, lagring og håndtering av importerte partier av oljeraps MON 88302 vil kunne medføre utilsiktet frøspill og tap av spiredyktige frø. Etablering av forvillede rapsplanter på lokaliteter der glyfosat regelmessig benyttes til ugraskontroll, eksempelvis langs jernbanelinjer og vegkanter, kan ikke utelukkes. Ferale rapsplanter med opphav i frøspill kan teoretisk representere et potensiale for utkryssing og spredning av transgener til dyrkede sorter og viltvoksende populasjoner i Norge. Forekomsten av transgene oljerapsplanter med opphav i frøspill, og introgresjon av genetisk materiale fra forvillet raps til nærstående, ville arter vurderes imidlertid til å være lav i et importsenario. Med unntak av toleranse mot glyfosat vil eventuelle avkom fra slike kryssinger ikke ha høyere fitness eller være forskjellig fra avkom fra hybridisering med konvensjonelle sorter av oljeraps.

VKMs faggruppe for genmodifiserte organismer konkluderer med at det er lite trolig at genspredning fra eventuelle ferale planter av oljeraps vil resultere i etablering av transgene planter på landbruksarealer eller medføre effekter på miljø i Norge.

Miljørisikovurderingen av den genmodifiserte oljerapslinjen MON 88302 vil ferdigstilles og sluttføres av VKMs faggruppe for genmodifiserte organismer når endelig dokumentasjon fra søker foreligger.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in oilseed rape is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or “elite” line without losing any part of the preferred line’s existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC ₁ , BC ₂ etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translation(s) of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards)
CP4	<i>Agrobacterium</i> sp. strain CP4
<i>cp4 epsps</i>	Codon optimised coding sequence of the <i>aroA</i> gene from <i>Agrobacterium</i> sp. strain CP4 encoding CP4 EPSPS protein
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase protein from the <i>Agrobacterium</i> sp. strain CP4
CTP	Chloroplast transit peptide
DAP	Days after planting
DN	Norwegian Directorate for Nature Management (Direktoratet for naturforvaltning)
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission/Community
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase enzyme
ERA	Environmental risk assessment
<i>E</i> -score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organization
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population
fw	Fresh weight
fwt	Fresh weight tissue

GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practices
Glyphosate	Broad-spectrum systemic herbicide
GM	Genetically modified
GMP	Genetically Modified Plant
GMO	Genetically modified organism
GMP	Genetically modified plant
H	hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect resistance management
Locus	The position that a given gene occupies on a chromosome
LOD	Limit of detection
LOQ	Limit of quantitation
MALDITOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da
mRNA	Messenger RNA
MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin
Northern blot	Northern blot is a technique used in molecular biology research to study gene expression by detection of RNA or isolated mRNA in a sample
NTO	Non-target organism
Near-isogenic lines	Term used in genetics, defined as lines of genetic codes that are identical except for differences at a few specific locations or genetic loci
OECD	Organization for Economic Co-operation and Development
ORF	Open Reading Frame, in molecular genetics defined as the part of a reading frame that contains no stop codons
OSL	Overseason leaf
OSR	Overseason root
OSWP	Overseason whole plant
PCR	Polymerase chain reaction, a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA
Phenological growth stages in oilseed rape (BBCH) (Table 1, Appendix 1)	0: Germination 1: Leaf development 2: Formation of side shoots 3: Stem elongation 5: Inflorescence emergence 6: Flowering 7: Development of fruit 8: Ripening 9: Senescence
R0	Transformed parent
Rimsulfuron	Herbicide, inhibits acetolactate synthase
RNA	Ribonucleic acid
RP	Recurrent parent
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
SAS	Statistical Analysis System
SD	Standard deviation

Southern blot	Method used for detection of DNA sequences in DNA samples. Combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridisation
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as <i>Agrobacterium tumefaciens</i> and <i>Agrobacterium rhizogenes</i> . The bacterium transfers this DNA fragment into the host plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border and requires the <i>vir</i> genes of the Ti plasmid.
TI	Trait integration
U.S. EPA	United States Environmental Protection Agency.
Western blot	Analytical technique used to detect specific proteins in the given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. The proteins are then transferred to a membrane where they are stained with antibodies specific to the target protein.
WHO	World Health Organisation.

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Background

On 5 September 2011, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium an application (Reference EFSA-GMO-BE-2011-101) for authorisation of the herbicide tolerant genetically modified (GM) oilseed rape MON 88302 (Unique Identifier MON-88302-9-2), submitted by Monsanto Company under Regulation (EC) No 1829/2003.

The scope of the current application is for all uses as any other oilseed rape, with the exception of seeds and other plant-propagating material for cultivation in the EU;

- Food and feed, containing or consisting of MON 88302
- Food produced from GM plants or containing ingredients produced from GM plants and feed produced from MON 88302
- Products other than food and feed containing or consisting of GM plants with the exception of cultivation.

After receiving the application EFSA-GMO-BE-2011-101 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 30 March 2012, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. All MS comments submitted during the consultation period will be considered by three working groups of the EFSA GMO Panel and responses to each individual comment will be provided.

According to the applicant, MON88302 oilseed rape has been submitted for regulatory approval concerning cultivation or import in several other countries beside the European Union, amongst others the USA, Canada, Japan, and the Philippines. The event has not yet received an authorisation for cultivation or import (CERA 2012).

The Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Food Safety Authority and The Norwegian Directorate for Nature Management to carry out a preliminary environmental risk assessment of oilseed rape MON 88302 for food and feed uses, import and processing, and submit relevant scientific comments or questions to EFSA on the application EFSA-GMO-BE-2011-101.

The environmental risk assessment will be completed and finalized by the VKM Panel on Genetically Modified Organisms when requested additional/final information from the applicant is available.

Terms of reference

The Norwegian Scientific Committee for Food Safety (VKM) carries out independent risk assessments for the Norwegian Food Safety Authority (Mattilsynet) across the Authority's field of responsibility as well as environmental risk assessments of genetically modified organisms for the Directorate for Nature Management (Direktoratet for naturforvaltning (DN)).

The Norwegian Food Safety Authority

By way of letter from the Norwegian Food Safety Authority dated October 15 2010 (ref. 2010/195445) the Norwegian Scientific Committee for Food Safety (VKM), has been assigned to evaluate submissions sent to the European Commission under the Regulation (EC) 1829/2003. The Regulation concerns commercial approval of genetically modified organisms and their derivatives including processed non-germinating products, intended for use as or in food or feed. VKM is to evaluate any potential health risks of such products. In addition, VKM is requested to evaluate the potential risks of genetically modified plants (GMPs) to the Norwegian agriculture and/or environment, and whether they are relevant for cultivation in Norway. Depending on the intended use of the GMP(s), defined by the applicant, the environmental risk assessment will be related to import, transport, refinement, processing and cultivation. If the submission seeks to approve the GMP(s) for cultivation, VKM is requested to evaluate the potential environmental risks of implementing the plant(s) in Norwegian agriculture compared to existing varieties (e.g. consequences of new genetic traits, altered use of pesticides and tillage). The assignment covers both direct and secondary effects of altered cultivating practices.

In the case of submissions regarding cultivation, VKM is further requested to assess risks concerning coexistence of cultivars. The assessment should cover the potential spread of plant materials from GMP-crops to areas of non-GMP crops as well as wild populations of endogenous plants of the same or similar species outside the cultivated areas, in addition to development and progression of weed populations. Evaluation of suggested measures for environmental monitoring provided by the applicants, in general or specific, are not covered by the assignment from the Norwegian Food Safety Authority.

The Norwegian Directorate for Nature Management

By way of letter from the Directorate for Nature Management (DN) dated June 15 2011 (ref. 2008/4367 ART-BI-BRH) the Norwegian Scientific Committee for Food Safety has been assigned to evaluate the potential environmental risks related to submissions of approval for the release of GMOs, i.e. cropping, sent to the EU Commission under the Directive (EC) 2001/18 and Regulation (EC) 1829/2003, and that are relevant to the Norwegian Gene Technology act. The task of VKM includes establishing scientific enquiries and comments as well as initial environmental risk assessments related to the submissions. VKM is also requested to deliver finalised reports on environmental risks in conjunction with national completion of the submissions.

The basis for evaluating the applicants environmental risk assessments is embodied in the act relating to the production and use of genetically modified organisms (Norwegian gene technology act), regulation on the assessment of potential impact based on the Norwegian gene technology act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants (EFSA 2006, 2010, 2011a), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

According to the assignment from the Directorate for Nature Management, VKM is to focus on environmental risk within the EEA and specific risks to Norway in particular.

Risk assessments provided by VKM on all submissions concerning approval of cultivation within the EEA are requested to include the potential environmental risks of the product related to any changes in agricultural practices. The assignment covers assessment of direct environmental impact of the intended use of pesticides with the GMO under Norwegian conditions, as well as changes to agronomy and possible long-term variations in the use of pesticides.

The preliminary reports on environmental risks provided by VKM should also consider the applicants recommended general and/or specific measures for monitoring. When recommended specific measures for monitoring are provided by the applicant, VKM must determine if these recommendations are sufficient to disclose potential direct short term effects, as well as delayed and indirect long term effects. If no specific measures are suggested in the application, VKM must also evaluate whether or not specific measures are required.

In accordance with the assignments given by the Norwegian Food Safety Authority, and the Directorate for nature management, VKM will provide input on said submissions without specific requirements, to the EFSA GMO EXTRANet (initial input), with copies sent to both the Norwegian Food Safety Authority and the Directorate for nature management. Likewise, if no input or comments are made or submitted to EFSA on certain submissions, VKM will inform of this as well. The Norwegian Food Safety Authority also requests that it is made evident in the risk assessments provided by VKM whether or not the applicant has committed to the EFSA guidelines on risk evaluation of GMOs and their derived products for food and feed (EFSA 2006, 2010, 2011a).

VKM is further requested to follow up on EFSA's response and to consider whether the inputs by VKM to the EFSA GMO EXTRANet are appropriately preserved in EFSA's own assessments.

The submission EFSA/GMO/BE/2011/101, genetically modified oilseed rape MON 88302, was posted on the EFSA GMO Extranet on 30 March 2012. The VKM GMO Panel will in compliance with the letters of engagement prepare an environmental risk assessment of oilseed rape MON 88302. The evaluation will be implemented in light of the intended use and in accordance with the principles denoted in the EFSA guidelines on risk assessment of genetically modified plants and derived products for food and feed (EFSA 2006, 2010, 2011a).

Assessment

1 Introduction

The genetically modified oilseed rape MON 88302 (Unique identifier MON-883Ø2-9-2) was developed to provide tolerance to the herbical active substance glyphosate by the introduction of a gene coding for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from *Agrobacterium tumefaciens*, strain CP4 (CP4 EPSPS). Glyphosate is a non-selective herbicide and is normally phytotoxic to a broad range of plants. Its mode of action occurs by binding to and inactivating the EPSPS protein, which is a key enzyme in the shikimate pathway that leads to the biosynthesis of the aromatic amino acids tyrosin, tryptophan and phenylalanine (Dill 2005; Duke & Powles 2008). The disruption of this pathway and the resulting inability to produce key amino acids prevents growth and ultimately leads to plant death.

The CP4 EPSPS protein in oilseed rape MON 88302 is insensitive towards inhibition by glyphosate. This protein is similar to the native EPSPS found in wild-type plants, but is not inactivated by glyphosate thus allowing the crop to be protected from the recommended dosages of glyphosate (Dill et al 2010). The *cp4 epsps* gene naturally contains a single point mutation that switches the nucleotide guanine for cytosine, which in turn causes the amino acid alanine to be substituted for glycine.

MON 88302 has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2006, 2011a), the environmental risk assessment of GM plants (EFSA 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2006, 2011c).

The environmental risk assessment of the GM oilseed rape MON 88302 is based on information provided by the applicant in the application EFSA/GMO/BE/2011/101, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature, monitoring reports and other relevant data.

In line with its mandate, VKM emphasized that assessments of sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act, shall not be carried out by the VKM Panel on Genetically Modified Organisms. The GMO Panel has therefore not considered possible health and environmental effects of cultivation and processing of oilseed rape MON 88302 outside the EU/EEA area.

2 Molecular characterisation

MON 88302 was developed through *Agrobacterium*-mediated transformation of hypocotyls from oilseed rape variety Ebony utilizing plasmid vector PV-BNHT2672. PV-BNHT2672 contains one T-DNA that is delineated by Left and Right Border regions. The T-DNA contains the *cp4 epsps* coding sequence under the control of the *FMV/Tsf1* chimeric promoter, the *Tsf1* leader and intron sequences, and the *E9 3'* untranslated region. The chloroplast transit peptide CTP2 directs transport of the CP4 EPSPS protein to the chloroplast and is derived from CTP2 target sequence of the *Arabidopsis thaliana shkG* gene. After transformation and subsequent rounds of self-pollination, homozygous R2 plants containing only a single T-DNA insertion were identified resulting in production of glyphosate-tolerant canola MON 88302.

2.1 Information related to the genetic modification

Oilseed rape MON 88302 was transformed by *Agrobacterium*-mediated gene transfer technology of hypocotyls from the conventional oilseed rape variety Ebony. A disarmed strain of *Agrobacterium tumefaciens* was the intermediate host used to transfer the T-DNA of plasmid PV-BNHT2672 into rapeseed cells to produce MON 88302. PV-BNHT2672 contains one T-DNA region with the full *cp4 epsps* expression cassette. Following transformation, self-pollination breeding and segregation methods were used to produce MON 88302.

2.1.1 Description of the methods used for the genetic modification

The elements included in the PV-BNHT2672 plasmid vector are described in Table 1. PV-BNHT2672 is approximately 9.7 kb and contains one T-DNA region including the *cp4 epsps* expression cassette, which is delineated by Left Border and Right Border regions. The T-DNA contains the *cp4 epsps* coding sequence under the control of the *FMV/Tsf1* chimeric promoter, the *Tsf1* leader and intron sequences, and the *E9 3'* untranslated region. The plant insert sequences between left and right border is shown in Figure 1 and Table 1. The chloroplast transit peptide CTP2 directs transport of the CP4 EPSPS protein to the chloroplast and is derived from CTP2 target sequence of the *Arabidopsis thaliana shkG* gene. The recipient plant materials were hypocotyl segments of a conventional oilseed rape, Ebony, which were excised from dark grown seedlings of germinated seed.

Table 1. Summary of genetic elements in MON 88302.

Genetic Element ¹	Location in Sequence	Function (Reference)
5' Flanking Sequence	1-839	DNA sequence adjacent to the 5' end of the insertion site
B-Right Border Region ²	840-882	DNA region from <i>Agrobacterium tumefaciens</i> containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982); (Zambryski et al., 1982)
Intervening Sequence	883-952	Sequence used in DNA cloning
P-FM1/Tg1	953-1892	Chimeric promoter consisting of the promoter of the Tg1 gene from the <i>Arabidopsis thaliana</i> encoding elongation factor EF-1a (Axelos et al., 1989) and enhancer sequences from the 5S promoter from the fugu ¹ transgene (Richard et al., 1987)
L-Tg1	1893-2038	5' untranslated leader (exon 1) from the <i>Arabidopsis thaliana</i> Tg1 gene encoding elongation factor EF-1a (Axelos et al., 1989)
I-Tg1	2039-2669	Intron from the <i>Arabidopsis thaliana</i> Tg1 gene encoding elongation factor EF-1a (Axelos et al., 1989)
Intervening Sequence	2670-2669	Sequence used in DNA cloning
TS-CP2	2670-2897	Targeting sequence from the <i>shkG</i> gene encoding the chloroplast transit peptide region of <i>Arabidopsis thaliana</i> EPSPS (Hermann, 1993; Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS-cp4 epsps	2898-4263	Codon optimized coding sequence of the <i>cp4 epsps</i> gene from the <i>Agrobacterium sp.</i> strain CP4 encoding the CP4 EPSPS protein (Curry et al., 2001; Padgett et al., 1996)
Intervening Sequence	4264-4307	Sequence used in DNA cloning
T-EP	4308-4950	5' untranslated sequence from the <i>rolC2</i> gene of <i>Plasma sativae</i> encoding the <i>Rubisco small subunit</i> (Chen et al., 1984)
Intervening Sequence	4951-4993	Sequence used in DNA cloning
B-Left Border Region ²	4994-5287	DNA region from <i>Agrobacterium tumefaciens</i> containing the Left Border sequence used for transfer of the T-DNA (Baker et al., 1982; Zambryski et al., 1982)
3' Flanking Sequence	5288-6174	DNA sequence adjacent to the 3' end of the insertion site

¹ B: Border, P: Promoter, L: Leader, I: Intron, T: Targeting Sequence, CS: Coding Sequence, T: Transcription Termination Sequence, ² Superscripts in Left and Right Border Regions indicate that the sequences in MON 88302 were truncated compared to the sequences in PV-BRIGHT2671.

2.2 Information relating to the GM plant

2.2.1 Information on the sequences actually inserted/deleted or altered

2.2.1.1 Size and copy number of all detectable inserts

Southern blot analyses were used to determine the copy number and insertion sites of the integrated DNA. The entire oilseed rape genome was assayed with probes that spanned the complete plasmid vector to detect the presence of the insert as well as confirm the absence of any plasmid vector backbone sequences.

The numbers of copies and insertion sites of the T-DNA sequences in the oilseed rape genome were evaluated by digesting MON 88302 and the conventional counterpart genomic DNA samples with the restriction enzyme *Ase I* or the combination of restriction enzymes *Sal I* and *Sca I* and hybridizing Southern blots with probes that span the T-DNA. This was accomplished by using probes that were not more than 2.5 kb in length to ensure a high level of sensitivity. This high level of sensitivity was demonstrated for each blot by detection of a positive control added at 0.1 copies per genome equivalent. PCR and DNA sequence analyses complement the Southern analyses.

The organization and sequence of the elements within the MON 88302 insert was confirmed by DNA sequence analysis. PCR primers were designed with the intent to amplify two overlapping DNA amplicons that span the entire length of the insert and the associated DNA flanking the 5' and 3' ends of the insert (see Figure 1). The amplified PCR products were subjected to DNA sequence analyses. This analysis determined that the DNA sequence of the MON 88302 insert is 4428 bp long and is identical to the corresponding T-DNA sequence of PV-BNHT2672. Control reactions with the conventional counterpart DNA and no template DNA control did not generate any PCR products as expected.

According to the applicant the molecular characterization of MON 88302 by Southern blot analyses demonstrated that the T-DNA was inserted into the oilseed rape genome at a single locus containing one copy of the *cp4 epsps* expression cassette. No additional elements were detected other than those associated with the insert. Moreover, no plasmid backbone sequences were detected in the genome of MON 88302.

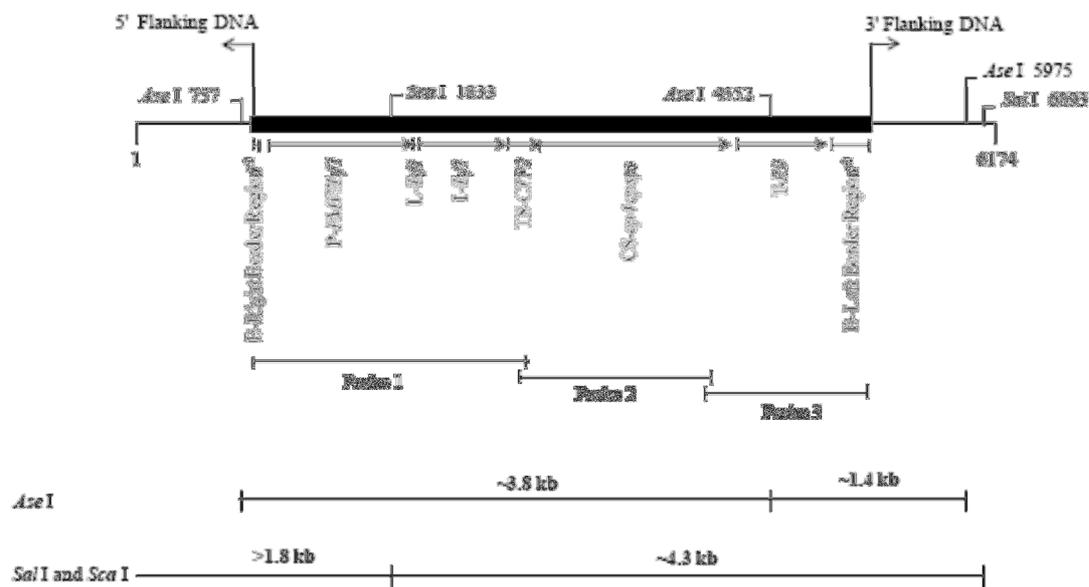


Figure 1. Schematic representation of the insert and flanking DNA in MON 88302.

The signal distribution justifies the conclusion that no major section of the T-DNA plasmid backbone is inserted in MON88302 oilseed rape.

2.2.1.2 Organisation and sequence of inserted genetic material at the insertion site

PCR and sequence analyses were performed on genomic DNA extracted from MON 88302 and the conventional counterpart to examine the MON 88302 insertion site. The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5' end of the insert paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure 1). The amplified PCR product from the conventional counterpart was subjected to DNA sequence analysis. DNA sequence analyses performed on MON 88302 determined the complete DNA sequence of the insert in MON 88302, confirmed the predicted organization of the genetic elements within the insert, determined the sequences flanking the insert, and examined the MON 88302 insertion site. Sequence analysis of the T-DNA insertion site indicated that a 9 base pair insertion immediately adjacent to the 3' end of the MON 88302 insert and a 29 base pair deletion from the conventional genomic DNA occurred during the insertion of the T-DNA into the conventional oilseed rape to form MON 88302. In addition, a single nucleotide difference between the conventional counterpart sequence and the known DNA sequence flanking the 3' end of the MON 88302 insert was also identified. This single nucleotide difference was most likely caused by single nucleotide polymorphism (SNP) segregating in the oilseed rape population (Trick et al. 2009).

The applicant has sequenced the entire insert and nearly 900 bp of flanking genomic DNA on each side of the insert. According to the applicant, the insert is 100% identical with the sequence of the inserted elements as they were present in the T-DNA plasmid.

No deletion was intended, however there was a 29 bp unintended deletion of DNA sequence at the site of cassette insertion in MON 88302. There is no known function associated with this deleted region, as revealed by the BLAST analyses and therefore it is not expected that it could affect the safety of the product. Minor deletions and/or insertions of DNA due to double-strand break repair mechanisms in the plant during *Agrobacterium*-mediated transformation process are not uncommon (Salomon & Puchta 1998).

The presence of MON 88302 insert in the nuclear genome is best shown by the Chi square analysis of the segregation results (Section A.2.2.4). The Chi square analysis of the segregation pattern, according to Mendelian genetics, was consistent with a single site of insertion into oilseed rape nuclear DNA.

The applicant concluded that the insertion locus of the large, functional insert in MON88302 is without any known function. It is apparently a short segment between two 3'-terminals of inversely oriented transcribed elements. The applicant has provided sufficient information to justify the conclusion that the insertion is located in the oilseed rape nuclear genome, and not in the chloroplast or mitochondrial genomes.

2.2.1.3 Sequence information for both 5' and 3' flanking regions and bioinformatics analyses on flanking regions and ORFs

A bioinformatics evaluation was performed to determine if any endogenous open reading frames (ORFs) or regulatory elements were disrupted by the insertion of the transferred DNA (T-DNA) present in MON 88302 or whether genes from the oilseed rape genome are present in the flanking genomic DNA adjacent to the T-DNA after transformation (Tu & Silvanovich 2011a). This evaluation was accomplished by submitting the DNA sequence flanking the insertion site of MON 88302 to a BLASTn and a BLASTx bioinformatics analyses. BLASTn is an alignment search that compares a nucleotide query sequence flanking the insertion site against a DNA sequence database. BLASTx is a sequence alignment search that compares conceptual translation products of a six-frame DNA query sequence against a protein database.

The databases used for the BLASTn analysis were the GenBank EST database (EST_2011) that includes 67 857 743 sequences, and the GenBank non-redundant nucleotide database (NT_2011) that

includes 14 564 296 sequences. BLASTx is a sequence alignment search that compares conceptual translation products of a six-frame DNA query sequence against a protein database. The database used for the BLASTx analysis was the GenBank non-redundant amino acid database (NR_2011) that includes 12 603 350 sequences. Results of this analysis confirm that it is unlikely that endogenous ORFs that encode protein sequences have been disrupted by the insertion of T-DNA in MON 88302.

The applicant has performed BLASTx analyses of all six possible reading frames (three on each DNA strand), and demonstrated that the 3' terminals of two likely transcriptional elements are located within approx. 60 bp upstreams to the 5' end of the large, functional T-DNA insert and approx. 30 bp. downstreams to the 3' end of this T-DNA. Neither of the two transcriptional elements was interrupted by the insert.

2.3 Information on the expression of the inserted sequence

2.3.1 Methods used and reference to raw data of CP4 EPSPS protein analysis

CP4 EPSPS protein expression levels were determined by a validated enzyme-linked immunosorbent assay (ELISA) in tissues collected from MON 88302. The ELISA method used was optimized to minimize method bias. Protein extracts from the test substance were analysed by ELISA with the appropriate protein standard and inter-assay negative and positive controls (Clark 2012a; Clark and Niemeyer 2010b).

The expression levels of the CP4 EPSPS protein were analysed from glyphosate treated and glyphosate untreated tissues of MON 88302. The tissues were obtained from field trials carried on in the US and Canada during the 2009 growing season (Clark, 2012a; Clark and Niemeyer, 2010b). According to the applicant, the field sites used for protein expression analysis were representative of oilseed rape producing regions and provide a range of environmental and agronomic conditions representative of commercial oilseed rape production.

2.3.2 The range and mean values for the levels of CP4 EPSPS protein

Seed tissues of MON 88302 were collected from four replicate plots planted in a randomized complete block field design during the 2009 growing season from three field sites in the US and in Canada. Seed samples of MON 88302 plants untreated and treated with glyphosate were analysed from each replicated plot at all field sites. Results from the protein expression levels in seed from MON 88302 are presented in Table 2. The mean CP4 EPSPS protein level determined across all sites from plots untreated and treated with glyphosate was 31 µg/g dw and 27 µg/g dw, respectively. The range of CP4 EPSPS protein levels in MON 88302 varied from 22 to 46 µg/g dw (Table 2).

The CP4 EPSPS protein levels (µg/g dw) determined from treated tissues of MON 88302 were comparable to those determined from untreated MON 88302 tissues, which indicates that glyphosate application in MON 88302 does not alter nor have any negative effects on the expression of the CP4 EPSPS protein in the plant.

Table 2. Developmental stage during tissue collection and summary of CP4 EPSPS protein levels in oilseed rape glyphosate treated and untreated seed from MON 88302 grown in US and Canadian field trials.

Tissue	Development Stage ¹	Days after planting (DAP)	CP4 EPSPS Mean (SD) Range) (µg/fw) ²	CP4 EPSPS Mean (SD) Range) (µg/dw) ³	LOQ/LOD ⁴ (µg/dw) ²
Seed treated	99	118-132	25 (5.2) 21-43	27 (5.6) 22-46	0.91/0.81
Seed untreated	99	118-132	29 (5.0) 20-40	31 (5.4) 22-42	0,91/0,81

¹ The oilseed rape development stage each tissue was collected. The oilseed rape growth stages are based on the Bayer, BASF, Ciba Geigy and Hoechst (BBCH) Cereal Grain Growth Scale (BBCH 2001).

² Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fw). The means, SD and ranges (minimum and maximum values) were calculated for each tissue across all sites. The numbers of samples (n) figured into the calculations are n=16.

³ Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dw). The dry weight values were calculated by dividing the µg/g fw by the dry weight conversion factor obtained from moisture analysis data.

⁴ LOQ=limit of quantification; LOD= limit of detection

The applicant shall provide documentation to demonstrate if the genetic modification has modified the levels of relevant endogenous protein(s), RNA(s) and/or specific metabolite(s). The data shall be provided from plants grown under conditions representative of typical cultivation practices, representing five or more generations or vegetative cycles. Where appropriate, the impact of specific treatments linked to the trait (e.g. use of herbicides) should also be assessed. The applicant has demonstrated that the large, functional T-DNA insert is expressed as intended and that its performance is not affected by treatment with glyphosate herbicide (the herbicide that the CP4-EPSPS protein provides tolerance for).

2.4 Genetic stability of the insert and phenotypic stability of the GM plant

2.4.1 Genetic stability of the insert in MON 88302

Genetic stability of the inserted DNA was investigated by Southern blot analysis of genomic DNA extracted from leaf tissues from four breeding generations of MON 88302, all of them produced by self-pollination (figure 2). The starting materials were seeds from Ebony and MON 88302 oilseed rape (generations R₂, R₃, R₄, R_{5a} and R_{5b}). Fifty seeds from each entry were planted. The breeding history of MON 88302 is presented in Figure 2. The specific generations tested are indicated in the legend of Figure 2. To analyse insert stability, additional samples from three generations of MON 88302 were evaluated by Southern blot analysis and compared to the R₃ generation.

According to the applicant the stability of the T-DNA present in MON 88302 across multiple generations was demonstrated by Southern blot fingerprint analysis. Genomic DNA from multiple generations of MON 88302 was digested with one of the enzyme sets used for the insert and copy number analyses and was hybridized with two probes that detect restriction segments that encompass the entire insert. This fingerprint strategy consists of two insert segments each containing its adjacent genomic DNA that assesses not only the stability of the insert, but also the stability of the DNA directly adjacent to the insert.

2.4.2 Phenotypic stability of the glyphosate-tolerance trait in MON 88302

A segregation analysis was conducted to determine the inheritance and stability of the T-DNA insert in MON 88302. During development of MON 88302, segregation data were recorded to assess the inheritance and stability of the coding sequence present in MON 88302. Chi-square (χ^2) analysis was performed over three generations to confirm the segregation and stability of the MON 88302 insert. The χ^2 analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 88302 breeding path for generating segregation data is described in Figure 3. The transformed R₀ plant was self-pollinated to generate R₁ seed. From the R₁ segregating population, an individual plant homozygous for the *cp4 epsps* coding sequence (subsequently designated MON 88302) was identified via TaqMan PCR copy number assay and Southern blot copy number analysis. The *cp4 epsps* homozygous R₁ plant was self-pollinated to give rise to R₂ plants that were self-pollinated to produce R₃ seed. At each generation, the homozygous plants were tested for the expected segregation pattern of 1:0 (positive: negative) for the *cp4 epsps* gene using a glyphosate spray test and/or TaqMan PCR assay.

A χ^2 analysis was performed on each of the F₂, F₃, and F₄ populations to compare the observed segregation ratio of *cp4 epsps* coding sequence to the expected ratio according to Mendelian principles of inheritance.

The Chi-square was calculated as:

$$\chi^2 = \sum [(o - e)^2 / e]$$

where o = observed frequency of the genotype or phenotype and e = expected frequency of the genotype or phenotype. The level of statistical significance was predetermined to be 5% ($\alpha = 0.05$).

Table3. Segregation of the *cp4 epsps* gene during the development of MON 88302.

Gen.	Total # Plants*	Obs. # Plants Homozyg. Positive	Obs. # Plants Hemizyg.	Obs. # Plants Homozyg. Negative	1:2:1 Segregation			X ²	P
					Expected # Plants Homozyg. Positive	Expected # Plants Hemizyg.	Expected # Plants Homozyg. Negative		
F ₂	220	51	122	47	55,00	110,00	55,00	2,76	0,2511
F ₃	166	39	94	33	41,50	83,00	41,50	3,35	0,1874
F ₄	198	53	97	48	49,50	99,00	49,50	0,33	0,8465

*Plants were evaluated for the copy number of the *cp4 epsps* gene using a real time TagMan PCR assay.

P=probability

The results of the χ^2 analysis of the MON 88302 segregating progeny are presented in Table 3. The χ^2 value in the F₂, F₃, and F₄ populations indicated no statistically significant difference between the observed and expected 1:2:1 segregation ratio (homozygous positive: hemizygous: homozygous negative) of *cp4 epsps* coding sequence. These results support the conclusion that the *cp4 epsps* expression cassette in MON 88302 resides at a single locus within the oilseed rape genome and is inherited according to Mendelian principles of inheritance.

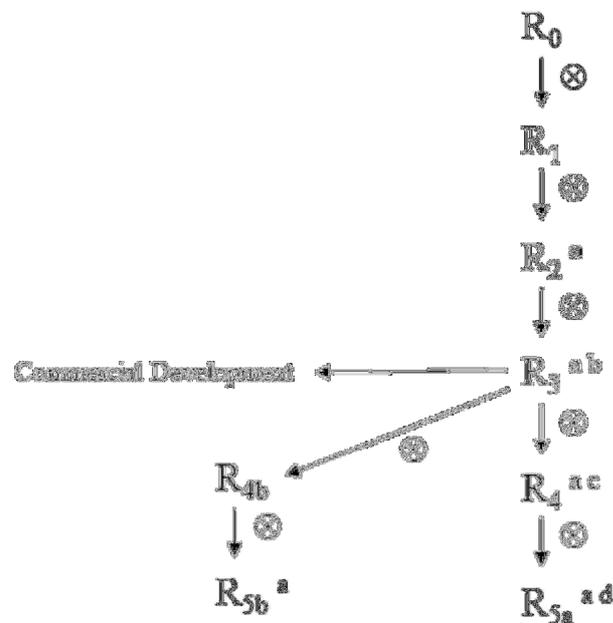


Figure 2. Breeding history of MON 88302

^a Generations used for generational stability of insert (Southern blot) and CP4 EPSPS expression (western blot).

^b Generations used for molecular characterization (Southern blot and DNA sequencing) and commercial development.

^c Generations used for protein expression analysis (ELISA), agronomic, phenotypic and environmental interactions analysis.

^d Generations used for protein expression analysis (ELISA) and compositional analysis (seed), seed dormancy and germination evaluation and pollen viability and morphology evaluation.

\otimes =self-pollination; R_0 =transformed plant

R_{5b} was propagated independently of R_{5a} beginning with the R_3 generation.

The stability of the T-DNA present in MON 88302 has been demonstrated across multiple generations by Southern blot fingerprint analysis. Additionally, segregation analysis was conducted to determine the inheritance and stability of the T-DNA insert in MON 88302. Results from this analysis demonstrated the inheritance and stability of the insert was as expected across multiple generations which corroborates the molecular insert stability analysis and establishes the genetic behaviour of the T-DNA at a single chromosomal locus.

The applicant has demonstrated that the novel protein CP4-EPSPS is functionally intact and stable over several generations. This is a strong indication of the functional stability of the insert.

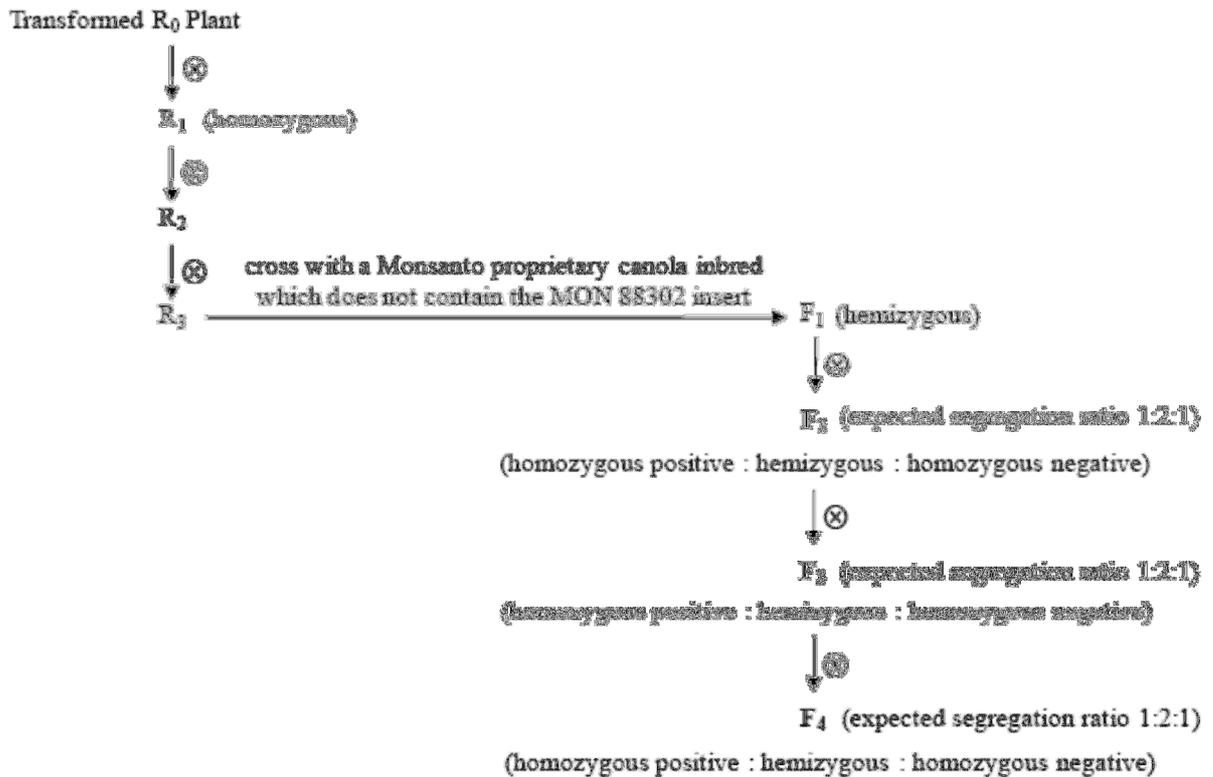


Figure 3. Breeding path for generating segregating data for MON 88302
An individual hemizygous plant from each of the F₁, F₂ and F₃ populations was self-pollinated to produce the population of the next generation. Chi-square analyses were conducted on segregation data from F₂, F₃ and F₄ populations.

2.5 Assessment based on available data

The VKM Panel on Genetically Modified Organisms finds that the insert and its inheritance pattern has been sufficiently described. We also find it justified that there is only one major T-DNA insert in MON88302 and that no major section of the T-DNA plasmid backbone is inserted.

3 Production, import and use of oilseed rape

Oilseed production

The worldwide production of oilseed rape in 2009 was about 31 million hectares (ha) (FAOSTAT 2009). The production is greatest in China (7.3 mill ha), India (6.3 mill ha) and Canada (6.1 mill ha). In Europe, oilseed rape was harvested from 8.5 million ha in 2009 (EU-27 6.5 million ha), with the greatest production in France, Germany, Britain and Poland. Total EU production of rapeseed in 2009 was approximately 21.4 million tonnes, while the estimate for the market year 2011/2012 is 18.8 million tonnes (Gain Report 2011).

The domestic production of oilseed rape is insufficient to cover the requirements of the EU, and imports have been increasing in recent years (SLF 2011). It is estimated that 3 million tonnes of rapeseed will be imported in 2011/2012, an increase of nearly 1 million tonnes from 2009/2010 (Gain report 2011). Most rapeseed imported to the EU originates from Ukraine and Australia.

In Norway, the acreage used for cultivation of oilseed rape has varied significantly during the past 15 years (SSB 2011). From 1996 to 2000, the total area used for cultivation of rapeseed varied between 60 and 70 thousand hectare. Signals from the Norwegian feed industry that larger quantities could be used than were being produced, resulted in the area used for rapeseed extent cultivation being increased to approximately 110 thousand ha. Following the peak years of 2001 and 2002, the domestic production of rapeseed was gradually reduced down to some 43 thousand ha in 2009 (Statistics Norway 2011). The decrease in area used for oilseed rape cultivation was primarily due to some years with relatively poor harvests (Abrahamsen et al. 2009, 2011). However, according to preliminary figures from Statistics Norway there has been an increase in oilseed rape cultivation over the past few years (59 thousand ha in 2010 and 52 thousand ha in 2011). Østfold and Akershus are the two most important regions for oilseed rape cultivation in Norway, being responsible for nearly 60 % of the total area.

Oilseed cultivation in Norway has traditionally been dominated by spring cultivars of turnip rape (*B. rapa* ssp. *oleifera*), and until 2003/2004 almost 90 % of the total area under cultivation of oilseed was sown with turnip rape. However, this production has significantly been reduced in recent years, and now accounts for about 50-60 % of the area. Oilseed rape has a growth period similar to late wheat cultivars (125-130 growing days) and is significantly later than turnip rape (about 155 growing days). Therefore it is primarily the counties around the Oslo Fjord that are recommended for rapeseed cultivation. The potential yield level from spring rapeseed is generally substantially higher than for turnip rape. While a good turnip rape yields 200 kg oilseed per ha, the rapeseed crop is as much as 300-400 kg oilseed per hectare (autumn sowing). The transition to almost half the crop now being spring rapeseed, having previously been almost exclusively spring turnip rape, has not been able to compensate for the reduction in area for oilseed cultivation. The area for winter rape depends largely on the possibility for sowing in early autumn and for overwintering. The cultivation area is normally very modest and accounts for less than 10 % of the total oilseed area (Abrahamsen 2011).

Import and applications

Development of oilseed rape varieties with a reduced content of toxic compounds has resulted in rape becoming one of the major oil and protein plants in this part of the world over the last decades. Using traditional selective breeding and mutagenesis, so-called "double low" or "double-zero" varieties have been developed with a modified fatty acid composition, in which the erucic acid content has been greatly reduced. Modern rape varieties contain less than 2 % erucic acid, while the content of oleic acid and linoleic acid has increased correspondingly. In addition, the glucosinolate content of the seed has been practically eliminated (< 25 µmol/g glucosinolate). For certain industrial applications, varieties with a high erucic acid content are generally preferred (Tamis & de Jong 2009).

Food

Before the introduction of erucic acid-free varieties, rapeseed oil was used only for industrial purposes. Today about 96 % of the rapeseed produced in Europe is used in the food industry. Rapeseed oil has a variety of uses in both the food industry and in households, including as cooking oil and in the manufacture of margarine, salad dressing, bakery items etc. (see Figure 1, Appendix 1).

The Norwegian imports of rapeseed oil in 2007 amounted to 1,136,431 tonnes (SLF 2008). With the exception of Norwegian company Matraps BA, there is no industrial processing of oilseed in Norway (G. Sandvik, SLF, pers. comm.). Norwegian Matraps BA was established in Østfold in 2001 and uses only Norwegian-produced raw material for the production of cold-pressed vegetable oil (M. Hoff, pers. comm.). The total production in 2010 was 207 tonnes of oil, derived from 1300 tonnes of rapeseed. This represents 43 % of the domestic rapeseed oil market. Other cooking oil on the Norwegian market is imported in bottles or in bulk for bottling in Norway.

The applicant maintains that processed oil is the only rapeseed product for human consumption. Tan et al. (2011), however, demonstrated that as rapeseed meal has a high biological value, with a balanced composition of essential amino acids and a superior amino acid profile compared with soya protein isolates, and also has good technological properties, there is considerable potential for the isolation of protein from rapeseed for use in the food industry and as an alternative to soy derivatives, milk, eggs and other plant-based and animal products. Several protein isolates from rapeseed have been approved by the U.S. Food and Drug Administration and received the status of "Generally Recognized As Safe (GRAS)", for use in foods (for example, U.S. Patent 7,611,735 B2, 2009).

According to the U.S. Canola Association, rapeseed is, amongst other uses, relevant as a protein supplement to acidic drinks such as sodas, sports drinks, and fruits juices. Furthermore, protein isolates from rapeseed can be used as emulsifiers and stabilisers in various food products and as a replacement for ingredients such as milk and eggs in foods such as biscuits, cakes, chocolate pudding, dressings, sauces, mayonnaise, protein bars, etc.

Feed

The proportion of marine oil used in fish-feed has been considerably decreased in recent years and replaced with vegetable oils. The most relevant plant-based ingredients in salmon feed are various products from soybean, rapeseed, wheat, maize, as well as palm oil and sunflower oil. According to Skretting's environmental report, 14.6 % rapeseed oil and between 5 and 10 % rapeseed meal was used in their salmon feed in 2010 (Skretting 2010). Otherwise, a maximum limit of 20 % rapeseed meal and 10 % rapeseed oil has been set for their use in feed for salmon and trout (OECD 2011).

The residues from oil-pressing are processed into livestock feed. Depending on the process employed these residues are referred to as "rapeseed (oil) cake" (from cold pressing) or "rape meal" (from hot pressing) (Tamis & de Jong 2009). These by-products are in high demand because of their high protein content and, in the case of cold pressing, high oil content. The crop residues left after the seed pods are harvested is known as rape straw and is likewise processed in the fodder industry. Rapeseed also serves as one of the raw materials for production of pet food, in particular seed mixtures for birds and rodents.

Due to the high performance requirements for livestock production, farmers are demanding ever more protein-rich feed types. This has led to a large increase in the import and use of protein ingredients such as rapeseed meal (SLF 2011). According to statistics from the Norwegian Agricultural Authority, 91 100 tonnes of processed rapeseed (pellets/meal) were imported in 2010 as a raw protein product for use in the Norwegian feed concentrate production (SLF 2011). Similarly, over 8 000 tonnes of oilseeds were imported for feed production. For comparison, 46 800 tonnes of rapeseed pellets and 7 600 tonnes of whole seeds were imported in 2007.

Rapeseeds are crushed and mixed into feed concentrate for ruminants, as with most of the domestic oilseed production. In 2010, 11 500 tonnes of Norwegian-produced oilseeds were used for the production of feed (SLF 2011).

Forage rape are used as green manure on arable farmland, as well as a foraging crop for livestock and in “wildflower mixtures” for verges and fields.

Other

Rapeseed oil is used in cosmetics and as a supplement or substitute for mineral oils in the chemical and engineering industries. Through esterification with methanol, rapeseed methyl ester (RME) has been produced, which has been in commercial use as biodiesel since the early 1990s.

Seed spillage

As oilseed rape seeds are small and round, they are easily lost during transport between fields and storage facilities. The extent of this seed dispersal has not been studied closely, but an investigation from the Netherland was conducted on the transport chains of potential GM crops, in particular oilseed rape, with a focus on spillage of seed in the environment (Tamis & Jong 2009). The study is based on qualitative information about when, where, and how much spillage occurred in the transport chains.

The rapeseed is brought onshore by coaster or inland barge and unloaded to a storage depot. While most oilseed rape seed is imported by boat and crushed in or near the ports of entry in the EU, a fraction of it can be transported inland to small independent crushing facilities by boat, truck or railway (Devos et al. 2009). The main points where losses of rapeseed occur are during quayside loading, overland transport to storage facilities and disposal of seed-cleaning waste. The greatest losses of imported rapeseed are probably associated with bulk transshipment prior to the transport to the processing plant, i.e. at quayside facilities and storage depots. A smaller fraction of losses will probably occur along the roadside during transport from port to processing plant (Tamis & Jong 2009).

According to Tamis & Jong (2009), the bulk of seed imported for oil pressing in the Netherlands enters a closed processing system in which the only environmental risk presented is from seeds escaping to the environment during transport to the crushing plant. Since all processing of oilseed for food uses in Norway are based on domestic rapeseed, this is not relevant in the Norwegian contexts.

The processing of rapeseed in the feed concentrate production, by contrast, does involve a greater environmental risk of seeds escaping to the wild, especially if seed mixtures are subsequently strewn outdoors. In addition, there is spillage of seeds along the transport chain from quayside to storage silo to truck/railway to the crushing plant. In addition, disposal of seed-cleaning residues and waste arising during process changes, and the presence of viable seeds in the meal or cake from the crushing process may result in seed spillage. According to the study, estimates of rapeseed losses along the transport chain range from 0.1-0.3 percent to 2-3 percent. A conservative estimate of 0.1 percent spillage for 2010, would therefore imply a total of 8 tonnes of oilseed rape seeds ending up in the environment in Norway per year, assuming an annually import of 8 000 tonnes whole rapeseeds for production of concentrate feeds (rapeseed pellets, meal and cakes not included).

4 Comparative assessment

4.1 Choice of comparator and production of material for the compositional assessment

Experimental design

The application EFSA/GMO/BE/2011/11 for food and feed uses, import and processing of oilseed rape MON 88302 within the European Union, presented compositional data from seed and forage material collected in field trials in the United States and Canada in 2009 and in Chile in the growth season 2009/2010. These field trials compared the composition of MON 88302 with a conventional counterpart having a comparable genetic background. The comparator included in the field trials was the commercial oilseed rape variety Ebony, which was used as the recipient for the DNA insertion to establish transformation event MON 88302. The breeding diagram of MON 88302 is shown in Figure 2, indicating the relationship between the GM plant and the conventional counterpart (R0). EFSA Regulation (EC) No 1829/2003 defines a conventional counterpart as „a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use“ (Art. 2.12). In line with this legal requirement the EFSA GMO Panel provides details on the criteria for the selection of appropriate comparators, under different scenarios, in the EFSA Guidance for the Selection of comparators for the risk assessment of GM plants (EFSA 2011b).

Several conventional commercial reference varieties were also included in the comparative assessments to provide a range of comparative values that are representative of existing conventional reference varieties for each measured compositional, phenotypic or agronomic characteristic. The commercial varieties used in these studies were selected to represent a range of genetic backgrounds and phenotypic characteristics and have been grown in the oilseed rape production regions. According to the applicant, they also reflect a range of data on natural variability within commercial oilseed rape varieties and therefore can provide context for interpreting experimental results. A total of seven different commercial reference varieties were used in the USA and Canadian field trials, while 12 (in agronomic and phenotypic studies) and 13 (in compositional studies) different varieties were used in the Chilean field trials.

The field trials were performed at five separate sites in North America (two field sites in the US (Minnesota and North Dakota) and three field sites in Canada (Manitoba and Saskatchewan), and four field sites in Chile (Maipo and Cachapoal). All the experimental locations were representative of oilseed rape cultivation areas of the countries. Field locations were acceptable environments for oilseed rape growth and are distributed across a wide geographical area to provide a variety of agronomic practices, soils and climatic factors

At each trial site, oilseed rape MON 88302, the conventional counterpart and the reference varieties were planted following a randomized complete block design with four replicates per site. At all sites, each plot was planted with four passes of a seed drill, where seeder pass 3 (alt 2) was designated for the collection of the phenotypic and environmental interactions data. Plots were separated by a 9-10 m conventional rape seed buffer in order to limit edge effects.

Prior to planting, each site prepared a proper seed bed according to local agronomic practices which could include tillage, fertility and pest managements practices. Each field location was scouted for agronomic and pest management needs including pest arthropods, diseases and weeds. Fertilizer, irrigation, agricultural chemicals and other management practices were applied as necessary. All maintenance operations were performed uniformly across the entire study area.

Glyphosate was applied to predetermined plots of glyphosate-treated MON 88302 at each site. The application was made to all 4 seeder passes at the 4 to 5 true leaf growth stage. The glyphosate

treatment was applied at approximately 1.8 kg a.e./ha (kilograms of acid equivalent per hectare). The agronomic/phenotypic analyses were carried out from the same fields than the compositional analyses.

Statistical analysis

According to the applicant, comparative plant characterization data between the GM crop and the control were considered in the context of evaluating potential contributions of the inserted trait to increased plant pest/weed potential. For each analysed characteristics, data were interpreted based on a statistical analysis conducted per EFSA statistical guidance, using both difference testing and equivalence testing (EFSA 2010b, 2011b). Interpretation of the statistical analysis was based on different outcome types described within EFSA statistical guidance (EFSA 2011d) (Appendix 1, Figure 1). Results were interpreted in a step-wise process shown in Figure 2, Appendix 1. For any outcome type that may require or that does require further evaluation (Outcome Types 3 through 7; Figure 2), the outcome type is considered in the context of whether or not the change is outside of the known variation for the crop, and whether or not the change could increase plant pest/weed potential of the GM crop. Ultimately, a weight of evidence approach considering all characteristics and results is used for the final risk assessment including the assessment for increased plant pest/weed potential.

Agronomic and phenotypic data

Analyses of variance (ANOVA) was conducted according to a randomized complete block design using SAS® (Version 9.2) in a combined-site analysis in which the data was pooled across all sites. Difference and equivalence tests were conducted using statistical models consistent with EFSA guidelines (EFSA 2010, 2011b). Difference testing was performed at the 10% level of significance ($\alpha = 0.10$), and equivalence testing performed at the 5% level ($\alpha = 0.05$). Glyphosate-treated MON 88302 and glyphosate-untreated MON 88302 were compared to the conventional counterpart “Ebony” using difference tests, and to the commercial reference varieties using equivalence tests. Statistical analysis was conducted for early stand count, days-to-first flowering, seed maturity, lodging, plant height, pod shattering, seed moisture, seed quality, yield and final stand count

Environmental interaction data

Glyphosate-untreated MON 88302 was also compared to the conventional counterpart for qualitative environmental interaction data, including plant response to abiotic stress, disease damage and arthropod damage. Environmental interaction data are categorical and were not subjected to statistical analysis. Glyphosate-untreated MON 88302 and the conventional control were considered different in susceptibility or tolerance to an abiotic stressor, disease, or arthropod pest on a particular observation date if the range of injury severity to MON 88302 did not overlap with the range of injury severity to the conventional counterpart across all four replicates

Table 4. Phenotypic, agronomic and environmental interaction characteristics of MON 88302 measured during 2009 US and Canada field trials and 2009-2010 Chile field trials (Technical Dossier: McPherson & Ahmad 2012a)

	Characteristics	Evaluation Stage¹	Evaluation Description
Plant phenotypic and agronomic charact.	Early stand count	12-14	Mean number of emerged plants from 3 separate one-meter long segments of the seeder pass
	Days to first flowering	60-69	Number of days after planting when 50% of the plants in a plot had one or more flowers
	Seed maturity	80-89	Number of days after planting when 30% of the seed in the lower 1/3 of the main raceme had changed from a green colour to black/brown/tan colour
	Lodging	80-89	A rating scale of 1-9, where 1=completely upright plants and 9=completely flat
	Plant height	71-89	Distance in cm from the soil surface to the top of the main raceme of 18 rep plants per plot
	Pod shattering	83-89	A rating scale of 1-9, where 1=0 to 10% shatter, and each subsequent value on the scale increasing in 10% increments up to 9=greater than 80% shatter
	Seed moisture	99	Percent moisture content of harvest seed
	Seed quality	99	At harvest, the percentage of green seeds from a 100 seed subsample from each plot
	Yield	99	Seed yield in tons per hectare and standardized to 8 % moisture
	Final stand count	Post-harvest	Mean number of plants from 3 separate one-meter long segments of the seeder pass rows at the time of harvest
Plant environm. interaction	Plant response to abiotic stress	4 x per season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0=no symptoms and 9=severe symptoms
	Disease damage	4 x per season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0=no symptoms and 9=severe symptoms
	Arthropod-related damage	4 x per season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0=no symptoms and 9=severe symptoms

4.2 Agronomic traits and GM phenotype

During field trials conducted over two seasons and different locations, phenotypic and agronomic data related to dormancy and germination, emergence and vegetative growth, reproductive growth and yield characteristics were collected. A description of evaluated phenotypic and agronomic characteristics and the designated developmental stages when evaluations occurred are listed in Table 4. In addition, the applicant has presented observational data from studies of plant environmental interactions four times during the growing seasons. The evaluations of environmental interactions include plant response to abiotic stressors (e.g. drought, wind, nutrient deficiency, etc), disease damage and arthropod damage (Table 4).

4.2.1 Agronomic and phenotypic results

Glyphosate untreated MON 88302

In the combined-site analysis, differences between glyphosate untreated MON 88302 and the conventional counterpart, and equivalence between the GM plant and a range of conventional commercial reference varieties were evaluated for 10 phenotypic characteristics (Table 5). Results of the statistical analyses for seven of the parameters observed (i.e. early stand growth, plant height, pod shattering, seed moisture, seed quality, yield and final stand count) were classified as “Outcome Type 1, equivalence category (I)”, which indicates no statistical differences between glyphosate-untreated MON 88302 and the corresponding conventional control, and equivalent to the commercial reference varieties.

Results from the analyses of lodging and seed maturity were classified as “Outcome Type 2, equivalence category (I), which indicates a statistically significant difference between glyphosate-untreated MON 88302 and the conventional counterpart ($p < 0.01$) and equivalence to the commercial reference varieties ($p > 0.05$) (Table 5). MON 88302, untreated with glyphosate, had less lodging (rating 2.3 vs. 3.1) and delayed seed maturity (107.1 vs. 103.6 days) compared to the conventional control. The values for untreated MON 88302 with respect to both lodging and seed maturity were, however, within the equivalence limits of the reference varieties included in the field trials.

The outcome from the combined-sites analyses of the variable “days to first flowering”, was classified as “Outcome Type 7, equivalence category (IV)”, and indicates a significant difference between untreated MON 88302 and the conventional counterpart Ebony ($p < 0.01$) and non-equivalence to the commercial reference varieties ($p < 0.05$). Glyphosate-untreated MON 88302 reached first flowering approximately four days later than the conventional control (63.0 vs. 58.7 days), and the mean value for this parameter was outside the equivalence limits calculated for the reference varieties (50.4-59.7 days). The as the measured endpoint for this parameter was within known variation for field grown canola as reported by the Canola Council of Canada (typical range of 54-69 days to first flowering), the applicant argued that the observed difference between MON 88302 and its comparator is unlikely to be of biological significance. It is not likely that an increase in “days to first flowering” would not contribute to increased plant pest/weed potential of the GM oilseed rape.

Glyphosate treated MON 88302

The outcome of the corresponding statistical analysis of glyphosate-treated MON 88302, the conventional counterpart and reference varieties for the 10 phenotypic characteristics shows the same results as the analyses of untreated MON 88302 (Table 6).

The statistical analyses of the field evaluations support that no phenotypic changes indicative of increased plant/pest potential of oilseed rape MON 88302 compared to conventional oilseed rape. Furthermore, the statistical analyses demonstrate that in-crop applications of glyphosate herbicide do not alter the phenotypic and agronomic characteristics of MON 88302 compared to conventional oilseed rape.

Table 5. Summary of phenotypic equivalence and difference tests on glyphosate untreated MON 88302 (Technical Dossier: McPherson & Ahmad 2012a)

Phenotypic characteristics (units)	MON88302	Control	Equivalence Limits ¹		Outcome ²	
	LS Mean ³	LS Mean ³	Lower	Upper	Equivalence Category	Type
Early stand count (# plants/linear m)	23,8	22,9	14,8	29,4	I	1
Days to 1. flowering (days after plant.)	63,0*	58,7	50,4	59,7	IV	7
Seed maturity (days after pl.)	107,1*	103,6	88,8	112,8	I	2
Lodging (1-9)	2,3*	3,1	1,5	6,0	I	2
Plant height (cm)	128,4	130,1	99,4	161,4	I	1
Pod shattering (1-9)	1,3	1,5	-0,1	3,1	I	1
Seed moisture (%)	7,8	7,9	5,8	10,8	I	1
Seed quality (%)	0,7	0,7	-1,0	1,7	I	1
Final stand count (# pl/linear m)	20,5	20,0	13,0	24,8	I	1
Yield (t/ha)	1,9	2,0	1,2	3,7	I	1

Table 6 Summary of phenotypic equivalence and difference tests on glyphosate treated MON 88302 (Technical Dossier: McPherson & Ahmad 2012a)

Phenotypic characteristics (units)	MON88302	Control	Equivalence Limits ¹		Outcome ²	
	LS Mean ³	LS Mean ³	Lower	Upper	Equivalence Category	Type
Early stand count (# plants/linear m)	23,4	22,9	14,8	29,4	I	1
Days to 1. flowering (days after plant.)	63,4*	58,7	50,4	59,7	IV	7
Seed maturity (days after pl.)	107,6*	103,6	88,8	112,8	I	2
Lodging (1-9)	2,3*	3,1	1,5	6,0	I	2
Plant height (cm)	130,7	130,1	99,4	161,4	I	1
Pod shattering (1-9)	1,4	1,5	-0,1	3,1	I	1
Seed moisture (%)	8,1	7,9	5,8	10,8	I	1
Seed quality (%)	0,6	0,7	-1,0	1,7	I	1
Final stand count (# pl/linear m)	20,0	20,0	13,0	24,8	I	1
Yield (t/ha)	2,0	2,0	1,2	3,7	I	1

4.2.2 Environmental interactions

According to the applicant, the assessed stressors observed in the field studies were at natural levels as no artificial infestation or imposed abiotic stress was used, and therefore, typically varied between observations at a site and among sites. Abiotic stress and disease damage data were collected from each plot using a 0-9 scale of increasing severity of observed damage.

Data were collected numerically and placed in the categories: none, slight, moderate or severe. These categorical data were not subjected to statistical analysis. The response of glyphosate-untreated MON 88302 and the conventional counterpart to an abiotic stress, disease and arthropod damage were considered different on a particular observation date at a site if the range of injury severity to glyphosate-untreated MON 88302 did not overlap with the range of injury severity to the control across all four replicates. For each observation at a site, the range of injury severity across the conventional reference varieties provided assessment data that are representative of commercial oilseed rape varieties.

Abiotic stress response

According to the applicant, no differences were observed between glyphosate-untreated MON 88302 and the conventional counterpart for any of the 24 comparisons of plant responses to abiotic stressors in the Chilean field trials (e.g. cold, compaction, drought, flood, frost, hail, heat, mineral toxicity, nutrient deficiency, wind) and 44 comparisons in the US and Canadian field trials (e.g. cold, drought, frost, heat, excessive moisture, wind), respectively (individual site assessments).

Disease damage

In an individual site assessment, no differences were observed between glyphosate-untreated MON 88302 and the conventional counterpart for any of the 24 comparisons for the assessed diseases in the Chilean field trials (e.g. *Alternaria*, *Phytophthora*, powdery mildew, *Sclerotinia*) and 42 comparisons in the US and Canadian field trials (e.g. *Alternaria*, *Sclerotinia*, black leg, downey mildew), respectively.

Arthropod damage

In an individual site assessment, no differences were observed between glyphosate-untreated MON 88302 and the conventional counterpart for any of the 23 comparisons for plant damage caused by arthropods in the Chilean field trials (e.g. aphids, cabbage worms, cutworms, grasshoppers, trips) and 48 comparisons in the US and Canadian field trials (e.g. aphids, armyworms, cutworms, grasshoppers, flea beetles), respectively.

4.2.3 Seed dormancy and germination

Oilseed rape dormancy is known to occur under field conditions and can contribute to seed persistence (Gulden et al. 2004a). According to the documentation from the applicant, a study aiming to assess the germination and dormancy characteristics of seed collected from MON 88302 has been conducted (McPherson 2010a, Technical Dossier, unpublished). In this study seed of MON 88302, its conventional counterpart Ebony and four commercial reference varieties, produced in the USA in 2009, were assessed for dormancy and germination characteristics using a standardized germination assay accepted by the Association of Official Seed Analysts (AOSA). Since two of the four reference varieties were genetically modified, a second study was performed, where the GM crops were excluded.

Germination boxes were arranged in environmental chambers in randomized complete block designs with four replications. Approximately 100 seeds of MON 88302, the conventional control and each of the four reference varieties were placed in separate germination boxes. The environmental chambers were programmed for 6 different temperature regimes (included AOSA-optimum treatment of 15/25 °C), where MON 88302 was compared to its comparator for percent normal germinated seed, percent abnormal germinated seed, percent dead seed, percent dormant seed and viable non-dormant (Table 7). Ungerminated seeds that remained after incubation at the five additional temperature regimes were moved to the AOSA-recommended temperature regime for final analysis. For all temperatures a reference range was calculated to provide seed germination and dormancy values representative of commercial oilseed rape.

No statistically significant differences ($p > 0.05$) were detected between MON 88302 and the conventional control for normal and abnormal germination and dead seed at any of the temperature regimes (Table 7). Table 7 shows the reference range calculated excluding the two GM varieties. However, exclusion of these varieties did not alter the study conclusions. No statistical differences were detected between MON 88302 and the control at 5 °C temperature regime for non-dormant seed. Analyses of variance was not conducted on dormant seed in all temperatures and viable non-dormant seed at the 15 °C, 25 °C, 30 °C, and 5/25 °C temperature regimes due to low numbers of seed in these categories (Table 7).

Table 7 Dormancy and germination characteristics of MON 88302 compared to conventional oilseed rape (Technical Dossier: McPherson 2010a, 2011a)

Temperature regime (°C)	Germination Category	Mean % (range)		Mean %
		MON 88302	Conventional control	Reference Range
5	Germinated	95.3 (94-98)	96.5 (95-98)	99.0-99.3
	Dead	2.3 (2-3)	1.8 (0-4)	0.5-0.5
	Viable non-dormant	2.5 (0-4)	1.8 (0-4)	0.3-0.5
	Dormant	0.0 (0-0)	0.0 (0-0)	0.0-0.0
15	Germinated	97.5 (95-100)	99.0 (98-100)	98.5-100.0
	Dead	2.5 (0-5)	0.8 (0-1)	0.0-1.5
	Viable non-dormant	0.0 (0-0)	0.3 (0-1)	0.0-0.0
	Dormant	0.0 (0-0)	0.0 (0-0)	0.0-0.0
25	Germinated	98.5 (97-100)	98.8 (97-100)	99.3-99.8
	Dead	1.3 (0-2)	1.0 (0-3)	0.3-0.8
	Viable non-dormant	0.3 (0-1)	0.3 (0-1)	0.0-0.0
	Dormant	0.0 (0-0)	0.0 (0-0)	0.0-0.0
30	Germinated	98.8 (97-100)	97.3 (95-99)	98.5-99.5
	Dead	1.0 (0-3)	2.8 (1-5)	0.5-1.5
	Viable non-dormant	0.3 (0-1)	0.0 (0-0)	0.0-0.0
	Dormant	0.0 (0-0)	0.0 (0-0)	0.0-0.0
5/25	Germinated	99.3 (98-100)	99.0 (98-100)	99.3-99.5
	Dead	0.8 (0-2)	1.0 (0-2)	0.5-0.8
	Viable non-dormant	0.0 (0-0)	0.0 (0-0)	0.0-0.0
	Dormant	0.0 (0-0)	0.0 (0-0)	0.0-0.0
15/25 (AOSA)	Normal germinated	98.0 (95-100)	98.8 (96-99)	96.8-98.5
	Abnormal germ.	1.3 (0-4)	1.3 (1-2)	1.5-2.0
	Dead	0.8 (0-2)	0.5 (0-2)	0.0-1.3
	Dormant	0.0 (0-0)	0.3 (0-1)	0.0-0.0

4.2.4 Pollen morphology and viability

Morphology and viability of pollen collected from MON 88302 compared to that of the conventional counterpart has been assessed by the applicant. Pollen was collected from glyphosate untreated MON 88302, the conventional control and four commercial reference varieties. All plants were grown in pots in growth chamber established at 21°C day/18 °C night with a 16 h photoperiod. The plants were arranged in a randomized complete block design with five replicates with one plant of each entry per replicate.

No statistical significant differences were detected between MON 88302 and the conventional control ($p>0.05$) ($n=5$) for percent viable pollen or pollen grain diameter (Table 8). Furthermore, according to the applicant, no visual differences in general pollen morphology were observed between MON 88302 and the conventional counterpart (data not shown). Introduction of the glyphosate-tolerance trait did not alter the overall morphology or pollen viability of MON 88302 compared to the conventional counterpart.

Table 8. Pollen viability and morphology evaluation of MON 88302 (Technical Dossier: McPherson 2011b)

Pollen Characteristics	Mean % (Range)		Mean %
	MON 88302	Conventional control	Reference Range
Viability %	99.8 (99.0-100.0)	100.0 (100.0-100.0)	98.0-99.3
Diameter (µm)	24.8 (22.5-27.7)	25.1 (22.4-28.1)	24.6-25.8

4.3 Assessment based on available data

Based on results from comparative analyses of data from field trials located at representative sites and environments in the USA, Canada and Chile, it is concluded that oilseed rape MON 88302 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of the herbicide tolerance conferred by the CP4 EPSPS protein. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON 88302 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glyphosate herbicide do not alter the phenotypic and agronomic characteristics of MON 88302 compared to conventional oilseed rape.

Evaluations of environmental interactions between genetically modified oilseed rape MON 88302 and the biotic and abiotic environment, and studies of seed dormancy, seed germination, pollen morphology and viability indicates no unintended effects of the introduced trait on these characteristics in MON 88302 oilseed rape.

5 Environmental risk assessment

The application EFSA/GMO/BE/2011/101 under Regulation (EC) No 1829/2003 is for the authorisation of genetically modified oilseed rape MON 88302 for import, processing and all uses as any other oilseed rape, excluding cultivation in the EU. Therefore, an environmental risk assessment (ERA) is performed in accordance with the principles of Annex II to Directive 2001/18/EC and following EFSA's Guidance on the ERA of GM plants.

Considering the intended uses/the scope of the application, excluding cultivation purposes, the environmental risk assessment is limited to indirect exposure through 1) accidental spillage of viable MON 88302 seeds into the environment during transport and processing; 2) manure and faeces of mainly animal fed with the GM oilseed rape; and 3) exposure through organic plant matter either imported or derived from by-products of industrial processes that used MON 88302.

5.1 Reproduction biology of oilseed rape

Oilseed rape (*Brassica napus* ssp. *oleifera* (DC.) Metzg) belongs to the *Brassicaceae* family, and is a member of the genus *Brassica*. Three major species of *Brassica* are grown commercially in Norway; *B. napus* (e.g. oilseed rape, swede), *B. oleracea* (e.g. cabbage, cauliflower, sprouts) and *B. rapa* (e.g. turnip and turnip rape). *B. napus* is an allotetraploid species with chromosome $2n = 38$, AACCC, originating from a interspecific hybridization between the two diploid species *B. oleracea* L. ($2n = 18$, CC) and *B. rapa* L. ($2n = 20$, AA) (OECD 1997/2011).

B. napus is mainly a self-pollinating species, but has entomophilous flowers capable of both self- and crosspollination (Treu & Emberlin 2000). The level of out-crossing varies depending on the availability of insect pollinators, variety and weather conditions. In fields, the average rate of out-crossing between adjacent plants is estimated to be approximately 30 %, but out-crossing rates between 12 to 55 % have been reported (Beckie et al. 2003; Pascher et al. 2010).

The pollen from oilseed rape can be transferred from plant to plant through physical contact between flowers of neighbouring plants and/or by wind and pollinating insects (Eastham & Sweet 2002; OECD 2011). The relative importance of wind versus insect pollination is unclear and probably varies with location and weather. The rape pollen grains have features that are typical of insect pollination being relatively large (32-33 μm), heavy and sticky (OECD 2011; Treu & Emberlin 2000). The flowers of oilseed rape produce nectar with relatively high concentrations of sugars and have a colour and structure which makes them attractive to insects, particularly bees. Honeybees (*Apis mellifera*) are an important insect pollinator of oilseed rape in Scandinavia, followed by bumblebees (*Bombus* sp.), solitary bees and Brachycera (Tolstrup et al. 2003; VKM 2007). Studies under natural conditions indicate a gradual decrease in pollen viability over 4 to 5 days (Ranito-Lehtimäki 1995, ref. Eastham & Sweet 2002). However, under ideal conditions *Brassica* pollen can be stored for up to 4 or 5 weeks without complete loss of viability.

Seeds are a major source of gene flow in oilseed rape. Oilseed rape shed seeds easily especially at harvest, with harvest losses estimated to 5-10 % of the average yield (Gulden et al. 2003, Gruber et al. 2004; Lutman et al. 2005). The rapeseeds are small (typical seed weight range 2.5-5.5 g/1000 seeds) and round, and are easily lost during the import, transportation, storage, handling and processing of oilseed rape commodities.

Endogenous (primary) dormancy does not occur in ripe seeds of oilseed rape (Pekrun et al. 1998). However, secondary dormancy can be induced under certain environmental conditions (long exposure to darkness, elevated temperatures, osmotic stress and sub-optimal oxygen supply) (OGTR 2002; Devos et al. 2012). Several studies have shown that genotype is the principal factor controlling the

potential for secondary dormancy in *B. napus* (Gulden et al. 2004a; Pekrun et al. 1997; Gruber et al. 2004).

Numerous studies have evaluated the persistence and secondary dormancy in the seed of different spring and winter oilseed rape cultivars, showing that oilseed rape seed can remain in secondary dormancy for many years in the soil seedbank, and germinate in subsequent years. Under field conditions, the persistence of secondarily dormant rape seed has been confirmed to be up to 5 years, and possibly up to more than 10 years in undisturbed soil (Lutman et al. 2003, 2005, Jørgensen et al. 2007; Messéan et al. 2007; D`Hertefeldt et al. 2008; Beckie & Warwick 2010).

Most of the seeds of oilseed rape, if left on or near the soil surface, will germinate and be killed by frost or cultivation or be eaten by rodents, birds and insects. Nevertheless, a small proportion may not germinate and secondary dormancy may be induced, particularly if the seed is buried. Studies have shown that at shallow burial depths, oilseed rape exhibit low seed bank persistence (Pekrun et al. 1998; Gulden et al. 2003). In an European study with winter oilseed rape, seeds buried immediately after seed shed, 30 % of the seed bank survived one winter compared to only 0.1 % when seeds were left on the undisturbed soil surface (Pekrun & Lutman 1998). At 10 cm depth, Gulden et al. (2004b) reported that seed bank populations shifted from a germinable to an ungerminable state and no seedling recruitment was observed. However, recently dormant oilseed rape seed has been found in non-till systems, indicating that seed can fall dormant on the soil surface, and need not to be buried in the dark (Gruber et al. 2010).

5.2 Unintended effects on plant fitness due to the genetic modification

In natural (undisturbed) ecosystems oilseed rape is not considered to be invasive or even a significant component of any natural plant community (OECD 2011), and generally its abilities to spread and establish outside cultivated areas in northern Europe are limited (Tolstrup et al. 2007).

Although oilseed rape has several properties that are characteristic of weed species, such as high reproductive capacity, rapid growth, and various mechanisms for pollination (self-pollination, airborne pollination, insectborne pollination), oilseed rape also has many characteristics that are typical of domesticated species, such as low genetic diversity, limited persistence, lack of primary seed dormancy, and limited capacity to compete with perennial species (Hall et al. 2005). Nevertheless, demographic studies of feral oilseed rape have shown the ability of oilseed rape to establish self-perpetuating populations outside agricultural areas, mainly in semi-natural and ruderal habitats in different countries in Europe, and in Canada and New Zealand (reviewed by Devos et al. 2012).

As with many annual weed species, oilseed rape is generally regarded as opportunistic species and can take advantage of disturbed sites due to its potential to germinate and capture resources rapidly. The species mainly establish on habitats that are continually disturbed, e.g. the margins of fields, roadside verges, railway lines, wastelands, docks etc., where the plants are exposed to minimal competition from perennial plants, especially perennial grass species (Claessen et al. 2005a, b).

In Norway, escaped oilseed rape plants are occasionally found near mills and dumping grounds as far north as Finnmark (Lid & Lid 2005; NBF 1999). Although the species can reproduce and survive for one generation without cultivation, it does not appear to have yet established permanent populations in Norway (Lid & Lid 2005; VKM 2007).

Studies of the potential for invasion by feral populations of oilseed rape into semi-natural and natural habitats outside cultivated areas indicate a substantial turnover of populations of feral oilseed populations: only a small percentage of populations occur at the same location over successive years, whereas the majority appears to die out rapidly (Crawley & Brown 1995, 2004; Elling et al. 2009; Nishizawa et al. 2009; Schafer et al. 2011). If habitats are disturbed on a regular basis by

anthropogenic activities, such as mowing, herbicide applications or soil disturbance, or natural occurrences, such as flooding, then feral populations can persist for longer periods (Claessen et al. 2005a; Garnier et al. 2006). The underlying ecological processes associated with the establishment and persistence of such populations has, however, rarely been investigated (Pivard et al. 2008a).

Because feral oilseed rape plants are more prevalent in areas with a high degree of oilseed rape cultivation (Squire et al., 2011), along roadsides (Crawley & Brown 2004; Knispel & McLachlan 2010), and near facilities for the handling, storage and processing of oilseed rape (Yoshimura et al. 2006; Peltzer et al. 2008) repeated spillage of seeds from both agricultural areas and from transport have been considered to be the main reasons for persistent populations of overspill oilseed rape. Several studies also conclude that feral oilseed rape populations are dependent on active seed dispersal (Sanvido et al. 2006).

However, several studies also indicate that oilseed rape is able to establish persistent populations outside areas of cultivation, which are not only dependent on annual seed dispersal, but also that persistence of the population is based on self-recruitment and contributions from the soil's seed bank. Pessel et al. (2001) found road side feral populations contained plants of old varieties that had not been grown for 8 to 9 years, indicating that the seed source was not entirely from recent vehicle spillage. Furthermore, between 35 and 40 % of these observed oilseed rape populations were not in areas of cultivation, and were shown to originate from the soil's seed bank, while under 10 % were related to local seed dispersal (Pivard et al. 2008). These results are in keeping with previous reports that seed of old rapeseed varieties can persist for at least 5 to 10 years after they were last reported grown (Squire et al. 1999; Orson 2002).

Results from the European research project SIGMEA show that there is little establishment of naturalised populations of oilseed rape plants outside of agricultural areas in northern Europe (Tolstrup et al. 2007). The project, which included studies of feral oilseed rape plants on roadsides, field margins, and waste lands in Denmark, Germany, UK and France (covering a total of 1,500 hectares and 16 years of observation), documented generally low frequencies of naturalised populations (on average, one population (1-10 plants) per km²). In the Danish study, 12 flowering plants/km² were recorded over two growing seasons. In France, the study was localised to areas with extensive oilseed rape cultivation, and showed significantly higher frequencies of escaped oilseed rape populations (15 populations/km²) (Lecomte et al. 2007).

The establishment of spontaneous oilseed rape populations, with both glufosinate ammonium (GA) and glyphosate tolerance, has been reported from harbour areas and along roadsides in Japan (Saji et al. 2005; Kawata et al. 2009; Nishizawa et al. 2009). As there has been no commercial cultivation of transgenic oilseed rape in Japan, it is assumed that this is related to seed spillage during transport of imported oilseed rape. Similar studies from British Columbia and Saskatchewan in Canada have shown that seed dispersal from regular transport has resulted in populations of herbicide-tolerant oilseed rape plants becoming established along railway lines and roads (Yoshimura et al. 2006). There are also equivalent reports from Germany, Britain, and France (ref. Nishizawa et al. 2010).

A study from USA reported an extensive distribution of persistent oilseed rape populations outside agricultural areas in North Dakota (Schafer et al. 2011). Populations were found both in habitats with selective pressures (roadsides sprayed with glyphosate) and habitats without obvious selective pressures. Of the oilseed rape samples analysed, 45 % contained the transgenes *cp4 epsps* or *pat*, while 0.7 % of plants expressed both CP4 EPSPS protein and PAT-protein. As there are no commercial oilseed rape cultivars with tolerance to both glyphosate and glufosinate on the market in USA, discovery of these combined traits in escaped populations confirms that there has been hybridization between different transgenic varieties. It is unclear whether this is due to pollen dissemination between fields with different transgenic cultivars and later spillage of seeds, or whether this is the result of crossing between resistant phenotypes of escaped plants outside cultivated areas. The highest densities of oilseed rape populations were found along highways, indicating establishment of escaped populations following seed spillage. Similar results have been reported from Canada (Knispel et al.

2008; Knispel & McLachlan 2010). Schafer et al. (2011) explains the distribution as being due to seed spillage during transport, but also points out that seed dispersal from fertile plants in escaped populations *in situ* contributes to the persistence of these populations.

Documentation of fitness, persistence, and invasive abilities of escaped populations of herbicide-tolerant oilseed rape plants are based on field trials, eco-physiological studies, and models, together with survey data (Devos et al. 2012). Field studies have confirmed that herbicide tolerance *per se* does not result in increased adaptation. In a three-year field trial in Britain, both conventional and transgenic oilseed rape cultivars with tolerance to glufosinate-ammonium were established in 12 locations with different environmental conditions (Crawley et al. 1993). Herbicides were not used in the study. The results gave no indication that the transgenic plants had increased invasive capacity of the existing plant communities, and also it was not demonstrated that herbicide-tolerance resulted in these cultivars being more invasive or persistent in disturbed habitats compared with conventional oilseed rape plants. In those cases where significant differences were discovered between transgenic and conventional cultivars, such as survival of seeds after burial in soil, the transgenic lines had, in all cases, reduced growth rates in comparison with the traditionally improved plant varieties. In a later study, Crawley et al. (2001) monitored conventional and transgenic (GA-tolerance) lines of oilseed rape, potato, maize, and sugar beet in 12 different habitats over a 10-year period. The results of this study demonstrated that the transgenic lines did not show better adaptation or increased persistence in comparison with the conventional varieties.

There is no evidence that tolerance to glyphosate or glufosinate-ammonium enhances seed dormancy, and thus the persistence of herbicide tolerant oilseed rape plants, compared with their corresponding, conventional comparators (Hails et al. 1997; Lutman et al. 2005; Messéan et al. 2007). Secondary dormancy in oilseed rape is shown to be more influenced by the genetic background of the parental lines than the presence of the herbicide tolerance traits (Lutman et al. 2003; Messéan et al. 2007). This indicates that herbicide tolerant oilseed rape is neither more likely to survive nor to be more persistent or invasive compared with its non-GM comparator. The herbicide tolerance trait can only be considered to be a selective advantage when the GM plants are sprayed with glyphosate- or glufosinate-ammonium containing herbicides. In addition, the ability of invasion of ruderal habitats also appears to be limited by areas for seed germination and competition from other vegetation.

It is therefore concluded that herbicide-tolerant oilseed rape does not have a greater capacity for survival, nor is it more persistent or have greater invasive abilities, compared with traditionally improved plant varieties. The ability to invade rural habitats appears to be limited by areas for seed germination and competition from other vegetation. Herbicide-tolerance can only be considered to be a selective advantage when the plants are sprayed with the relevant herbicides.

Field trials with the oilseed rape cultivar MON 88302 in representative areas for oilseed rape cultivation in USA, Canada, and Chile have shown equivalence between the transgenic line and the corresponding, unmodified control with respect to agronomic and phenotypic characteristics. With the exception of tolerance to glyphosate, according to the applicant, no evidence of significant differences with respect to the characteristics associated with reproduction and vegetative growth have been demonstrated in these field studies, between the oilseed rape cultivar and conventional varieties with equivalent genetic backgrounds. Investigations of interactions between the oilseed rape cultivar MON 88302 and biotic and abiotic factors, as well as studies of seed dormancy, seed germination, morphology, and pollen vitality, indicate no unintended effects of the introduced characteristics on the phenotypic characteristics of MON 88302.

In Norway, glyphosates are used to combat monocot species (especially couch-grass), and dicot seeding and perennial weeds, either before germination, before establishment, or after harvesting of all crops (www.plantevernguiden.no; Stenrød et al. 2007). Furthermore, glyphosate formulations are approved for use in ripe barley fields without companion crops, and for shielded spraying in orchards, on ornamental trees, and on ornamental and berry bushes. Pesticides with glyphosate are also used extensively against thicket growth in the forestry industry and for vegetation control along roads and

railway lines, in yards and industrial areas, and for protecting power lines, plant nurseries, etc. In recent years the railway industry has used 13,000-15,000 litres of Roundup annually along railway lines (Samferdsel og Miljø 2011). Widespread use of glyphosate for weed control outside agricultural areas might, however, result in selective advantages for MON 88302 along transport routes, in ports and besides processing plants.

In the period 2007-2011, the average annual usage of glyphosate in Norway was 301,000 kg active ingredient (Mattilsynet 2012). This represents about 55 % of the quantity of herbicides used. In comparison, the average usage of glyphosate in the period 1982-1986 was about 70 tonnes. The increased use can largely be attributed to changes in tillage practices. Reduced tillage (plough-free cultivation) results in a rise in annual, biennial, and perennial weed species compared with cultivation systems with autumn or spring ploughing, and therefore increases the requirement for plant protection measures (Stenrød et al. 2007).

5.3 Potential for gene transfer

A prerequisite for any gene transfer is availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via seed spillage followed by cross-pollination. Considering the scope of the application and the physical characteristics of oilseed rape seeds, possible pathways of dispersal are from: (1) occasional oilseed rape plants originating from indirect exposure through manure and faeces from gastrointestinal tracts of animals fed on GM oilseed raps; (2) accidental spillage of viable MON 88302 seeds into the environment during transport and processing for food and feed uses (including germination from an oilseed rape seed bank previously established by accidental release, and (3) exposure through organic plant matter either imported or derived from by-products of industrial processes that use MON 88302.

Exposure of microorganisms to recombinant DNA occurs during the breakdown of plant material on arable land and/or pollen in agricultural fields and in the field margins. Recombinant DNA is also a component of a variety of food and feed products derived from transgenic plant material. This means that micro-organisms in the digestive tract of humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic oilseed rape) may also be exposed to transgenic DNA.

Several species within the *Brassica* complex are related to oilseed rape and there are species in related genera that are either cultivated, or act as feral or wild populations in non-agricultural habitats in Norway. Possible vertical gene transfer will therefore be related both to cross-pollination of conventional and organic varieties, and to escaped and wild populations/species.

5.3.1 Plant-to-microorganism gene transfer

Experimental studies have shown that gene transfer from transgenic plants to bacteria rarely occurs under natural conditions and that such transfer depends on the presence of DNA sequence similarity between the DNA of the transgenic plant and the DNA of the bacterial recipient (Nielsen et al. 2000; De Vries & Wackernagel 2002, reviewed in EFSA 2004b, 2009b; Bensasson et al. 2004; VKM 2005).

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgenes present in in MON 88302 to unrelated species such as bacteria.

It is however pointed out that there are limitations in the methodology used in these experimental studies (Nielsen & Townsend 2004). Experimental studies of limited scale should be interpreted with caution given the scale differences between what can be experimental investigation and commercial plant cultivation.

Experiments have been performed to study the stability and uptake of DNA from the intestinal tract in mice after M13 DNA was administered orally. The DNA introduced was detected in stool samples up to seven hours after feeding. Small amounts (<0.1%) could be traced in the blood vessels for a period of maximum 24 hours, and M13 DNA was found in the liver and spleen for up to 24 hours (Schubbert et al. 1994). By oral intake of genetically modified soybean it has been shown that DNA is more stable in the intestine of persons with colostomy compared to a control group (Netherwood et al. 2004). No GM DNA was detected in the feces from the control group. Rizzi et al. (2012) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals.

The origin and properties of the inserted gene does not suggest a novel directional positive selection of the plant transgenes in MON 88302 in bacterial recipients.

In conclusion, the VKM GMO Panel consider it is unlikely that genes from MON 88302 will transfer and established in the genome of microorganisms in the environment or in the intestinal tract of humans or animals

5.3.2 Plant-to-plant gene flow

The potential for cross-pollination between oilseed rape cultivar MON 88302 and conventionally bred oilseed rape varieties, other cultivated *Brassica* species, related species, or overspill oilseed rape plants occurring as weeds in agricultural areas or in natural or semi-natural habitats, depends on the extent of accidental seed dispersal and the establishment of overspill plants in association with transport, storage, handling, and further processing. Several studies investigating gene exchange with related wild plants or other cultivated varieties or species of agricultural plants have been published. However, these studies are mostly related to the cultivation of oilseed rape, either in field trials or commercial fields for cultivation. Little data have been published that can elucidate the potential for spread and integration of transgenes from dispersed escaped plant populations or from populations under different environmental conditions.

5.3.2.1 Potential for cross-pollination with cultivated oilseed rape varieties

Studies of pollen dispersal and out-crossing in oilseed rape indicate that there is significant variation regarding dispersal and frequency of out-crossing. Dispersal potential depends on a number of factors, such as variety characteristics (fertility ratio/flowering synchrony), spatial arrangements of plants, relative size of the pollen donor and recipient populations, field and landscape features, the presence of pollen barriers, environmental conditions (temperature, wind speed and wind direction, humidity etc.), density of insect populations, etc. (Warwick 2004; Messéan et al. 2006). Different field experiments, with various experimental designs, locations, and environmental conditions, have shown that most of the pollen is transported less than 10 metres from the pollen source, and that the amount of pollen decreases sharply as the distance from the donor plants increases (Timmons et al. 1995, 1996; Thomson et al. 1999; Warwick 2004; NIAB 2006).

The majority of out-crossing occurs within the first 100 metres. Data from over 100 field trials with spring and winter oilseed rape in the British FSE-Project ('Farm Scale Evaluation') have been used to predict unintended introduction of transgenes into harvested seeds as a function of, among other factors, isolation distance and field size (length/width) (Weekes et al. 2005; NIAB 2006). The results from this study showed that when plants were used that contained two transgene copies, less than 0.3 % introduction was registered in conventional crop fields at distances of 35 metres, given a field depth of 200 metres. In those cases where pollen competition from the donor field was reduced by halving

the width of the field, the introduction increased by 0.6 % and 0.8 % for winter and spring oilseed rape, respectively. For comparison, a less than 0.4 % introduction was found when using hemizygotic plants in field widths of 100 metres.

However, several studies have shown that significant amounts of oilseed rape pollen can be transported over long distances by the wind and by insects. In a study of gene flow in herbicide-resistant oilseed rape between commercial crop fields in Canada, pollen dispersal of up to 800 metres from the pollen source was demonstrated (Beckie et al. 2003). Similarly, results from experiments in Britain and Australia have shown pollen dispersal ranging from 400 meters to 4 km from the donor plants (Scheffler et al. 1995; Timmons et al. 1995; Thompson et al. 1999; Rieger et al. 2002). With the potential for potential for pollen dispersal via long distance fliers, such as some bumblebees, honey bees, hover flies and pollen beetles, dispersal over distances of several tens of kilometres should be expected (VKM 2007).

Feral oilseed rape MON 88302 arising from spilled seed could theoretically pollinate conventional crop plants if feral populations are immediately adjacent to field crops, and shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops. However, the frequency of such events is likely to be extremely low. Squire et al. (2011) and Devos et al. (2012) concluded that this route of gene flow would not introduce significant numbers of transgenic plants into agricultural areas or result in any environmental consequences.

5.3.2.2 Potential for interspecific hybridisation and introgression with other *Brassica* species

Accidental seed spillage and the establishing of volunteer may also lead to unwanted gene flow via pollen and represent a potential for out-crossing between cultivated varieties and wild populations (Devos et al. 2004). In addition to hybridization with other cultivated varieties of oilseed rape and turnip rape, genetic exchange between oilseed rape and other cultivated forms and subspecies of *B. napus*, for example turnip (*B. napus* ssp. *rapifera*) and swede (*B. napus* ssp. *napobrassica*), is theoretically possible, although unlikely. Both turnip and swede are biennial plants that don't normally flower during the year of cultivation. There is no seed cultivation of forage rape in Norway and only negligible production of swede seeds.

There is several plant species that are related to *B. napus* that are either cultivated, occurs as weeds of cultivated and disturbed lands, or grow in the wild outside cultivation to which gene introgression from *B. napus* could be of concern. These are found both in the *Brassica* species complex and in related genera. The following closely related species are present to varying degrees in the Norwegian flora (Lid & Lid 2005); wild turnip (*B. rapa* ssp. *campestris* (L.) Clapham, black mustard (*B. nigra* (L.) W.D.J. Koch), mustard greens (*B. juncea* (L.)), hoary mustard (*B. adpressa* Boiss.), wild radish (*Raphanus raphanistrum* ssp. *raphanistrum*), annual wall rocket *Diplotaxis muralis*, perennial wall rocket (*D. tenuifolia* (L.) DC), field mustard (*Sinapsis arvensis* L.), white mustard (*Sinapsis alba* L.), common dog mustard (*Erucastrum gallicum* (Willd.) O.E.Schulz).

A large number of these species are, however, partly or completely isolated due to varying degrees of ecological and genetic barriers (Eastham & Sweet 2002; Devos et al. 2009; Jørgensen et al. 2009). A series of controlled crosses between *B. napus* and related taxa have been reported in the scientific literature, conducted under ideal experimental conditions (e.g. artificial pollination and embryo rescue techniques in laboratory). These relatives include *B. rapa*, *B. juncea*, *B. nigra*, *B. adpressa*, *R. raphanistrum*, *S. arvensis*, *E. gallicum* and *D. tenuifolia* (OECD 2011). Because of a mismatch in the chromosome numbers most hybrids have a severely reduced fertility (very low pollen viability and seed production), and only some of the interspecific embryos develop into viable seed. Exceptions are hybrids obtained from crosses between oilseed rape and wild turnip (*B. rapa* ssp. *campestris*) and mustard greens (*B. juncea*), where spontaneously hybridising and transgene introgression under field conditions have been confirmed (Mikkelsen & Jørgensen 1997; Xiao et al. 2009; OECD 2011).

Interspecific and intergeneric sexual crossing attempts, degree of success and potential for gene introgression with different species in the cruciferous family are presented in Table 9 (OECD 2011). A summary of some of these studies are presented in the following paragraphs and discussed in more details in the Appendix 2.

Table 9. Interspecific and intergeneric sexual crossing attempts, degree of success and potential for gene introgression¹ (Source: OECD 2011).

Interspecific cross	Sexual cross	Field cross	Seeds/cross	BC (male)	BC (female)	Potential		References
						Natur. cross	Introgression	
<i>Brassica napus</i>								
<i>B.napus x B. juncea</i> <i>B. juncea x B.napus</i>	Y Y	Y Y	4 0.54	Y Y	Y Y	H H	H H	Bing et al. 1991, 1996; Frello et al. 1995; Jørgensen et al. 1998, 1999
<i>B. napus x B. nigra</i> <i>B. nigra x B.napus</i>	Y	Y	0-0.09 0.01	Y F	F F	L VL	L L	Bing et al. 1991; Brown & Brown 1996; Daniels et al. 2005
<i>B. napus x B. oleracea</i> <i>B. oleracea x B. napus</i>	Y							Gupta 1997
<i>B. napus x B. rapa</i> <i>B. rapa x B. napus</i>	Y Y	Y Y	M M	Y Y	Y Y	H H	H H	Bing et al. 1991, 1996; Brown & Brown 1996; Gupta 1997; Jørgensen & Andersen 1994; Landbo & Jørgensen 1997; Mikkelsen et al. 1996;
<i>B. napus x B. adpressa</i> <i>B. adpressa x B. napus</i>	Y Y	Y Y	2	Y	Y	H	L	Lefol et al. 1991, 1995, 1996b; Eber et al. 1994; Chevré et al. 1996
<i>B. napus x B. tournefortii</i> <i>B. tournefortii x B. napus</i>	Y F	NR	0.69			L VL	L VL	Nagpal et al. 1996; Gupta 1997; Salisbury 2002
<i>B. napus x Diplotaxis muralis</i> <i>D. muralis x B. napus</i>	Y NR	NR NR	0.28			L	VL	Bijral & Sharma 1996a
<i>B. napus x D. eruroides</i> <i>D. eruroides x B. napus</i>	NR Y	NR NR		Y		VK	VL	Ringdal et al. 1987
<i>B. napus x Raphanus raphanistrum</i>	Y	Y	10 ^{-4,-8}	Y	Y	H	L	Darmency et al. 1998; Eber et al. 1994; Lefol et al. 1997; Rieger et al. 1999;

<i>R. raphanistrum</i> x <i>B. napus</i>	Y	F						Chevré et al. 1997a, 1998
<i>B. napus</i> x <i>R. sativus</i> <i>R. sativus</i> x <i>B. napus</i>	Y NR	NR	0					Gupta 1997; Ammitzbøll & Jørgensen 2006
<i>R. napus</i> x <i>Eruca sativa</i> <i>E. sativa</i> x <i>B. napus</i>	Y NR	NR NR				L	VL	Birjal & Sharma 1996b
<i>B. napus</i> x <i>Erucastrum gallicum</i> <i>E. gallicum</i> x <i>B. napus</i>	Y F	F NR	0.1 0	Y	Y	VL VL	VL VL	Lefol et al. 1997; Warwick et al. 2003
<i>B. napus</i> x <i>Sinapis alba</i> <i>S. alba</i> x <i>B. napus</i>	Y F	NR NR	Y			VL EL	VL EL	Chevré et al. 1994; Brown et al. 1997
<i>B. napus</i> x <i>S. arvensis</i> <i>S. arvensis</i> x <i>B. napus</i>	Y Y	F F	0.18 F	F		L EL	VL EL	Bing et al. 1991; Moyes et al. 2002; Sweet et al. 2007; Lefol et al. 1996b.

¹ Y=successful cross by hand pollination or in the field, F=Cross attempted but failed, NR=Not reported.
Probability of crossing in nature and/or gene introgression: H=High, L=Low, VL=Very low, EL= Extremely low

Wild turnip (*B. rapa* ssp. *campestris* (L.) Clapham)

A number of studies have shown that hybridization between *B. napus* and *B. rapa* ssp. *campestris* occurs spontaneously in the field (e.g. Jørgensen & Andersen 1994; Landbo et al. 1996; Mikkelsen et al. 1996; Jørgensen et al. 1996, 1998; Halfhill et al. 2004). Hybridization between these species can occur in both directions, but primarily arises with *B. rapa* ssp. *campestris* as the pollen donor. Natural interspecific hybridisation between *B. rapa* and *B. napus* varies widely, depending on cultivar characteristics, the environment under which the plants develop and the design of the experiment, particularly the ratio of *B. napus* and *B. rapa* plants. Transgene introgression is likely to take place when oilseed rape and wild turnip grow in close proximity over successive growing seasons, especially if no significant fitness costs are imposed to backcross plants by transgene acquisition (Snow et al. 1999). In Danish trials up to 95 % hybrids were found in *B. rapa* progeny (Mikkelsen et al. 1996), while studies from Canada (Bing et al. 1991) and England (Wilkson et al. 2000) reported less than 1 % hybridisation.

Interspecific hybrids between *B. napus* and *B. rapa* are mostly triploid, with reduced pollen fertility, and hence low ability to pollinate and form backcrosses with *B. napus* (Jørgensen & Andersen 1994; Andersson et al. 2010; Norris et al. 2004; Warwick et al. 2003). The survival rate of hybrid seedlings is also low (<2 % survival) (Scott & Wilkinson 1998), reducing the rate of introgression (Jørgensen et al. 1996). Introgression of HR transgenes from *B. napus* to *B. rapa* has occurred in Europe (Jørgensen et al. 1999; Hansen et al. 2001; Norris & Sweet 2002). Extensive introgression has e.g. been reported from a mixed population of *B. napus* and *B. rapa* in organically farmed fields in Denmark, 11 years after conversion (Hansen et al. 2001). Of 102 plants analysed, only one individual was a first generation hybrid (F₁-hybrid), while almost half of the plants had specific genetic markers from both *B. napus* and *B. rapa*. A UK study of naturally occurring wild turnip in GM oilseed rape also showed a high incidence of hybridization between these species (Norris et al. 2004)

The first report that documents the persistence and stable incorporation of transgenes from herbicide-resistant oilseed rape into *B. rapa* ssp. *campestris* in commercial cultivation fields was published in 2008 by Warwick et al. (Warwick et al. 2008). This study confirmed the persistence of a glyphosate tolerance trait over a period of 6 years in a population of *B. rapa* in the absence of selective pressure in the form of glyphosate treatment and in spite of fitness costs associated with hybridisation. This was demonstrated in both F₁-generations and backcrossed generations of the hybrid. Elling et al. (2009) measured the extent of hybridisation between autotetraploid *B. rapa* varieties (female) and *B. napus* (pollen donor) under experimental field conditions and found that the hybridisation with tetraploid *B. rapa* seemed to be more likely than with diploid *B. rapa*. The authors reported higher pollen fertility in these hybrids than those formed with diploid *B. rapa* and suggested that introgression frequencies from *B. napus* to *B. rapa* would be higher in tetraploid *B. rapa*. They also reported the presence of some feral tetraploid *B. rapa* populations in Germany, but did not report on interspecific hybrids or backcrosses in these populations. Surveys conducted in Japan did not detect transgenes in seed collected from wild relatives of *B. napus* (*B. rapa* and *B. juncea*) sampled at ports, and along roadsides and riverbanks (Saij et al. 2005).

Wild turnip is native to Norway. The species is a common weed in arable lowlands and is also widely distributed in the villages in the valleys and mountains in southern Norway and the most northerly counties (Lid & Lid 2005).

Mustard greens/brown mustard (*B. juncea* (L.) Czern.)

Hybrids have been produced by controlled crossings between oilseed rape and mustard greens (Mikkelsen & Jørgensen 1997). It is also known that the hybrids can form spontaneously under natural field conditions (Frello et al. 1995; Jørgensen et al. 1996; Liu et al. 2010). In a Danish study, Jørgensen et al. (1996) reported a 3 % hybridization frequency from crossings with *B. napus* as a pollinator. Equivalent results have been reported from Canada (Bing et al. 1991; Eastham & Sweet 2002). Species hybridization can occur in both directions, but is most successful with *B. napus* as the pollen donor. The F₁-hybrid has low fertility (0 – 28 %), but expression of transgenes has been observed in the first generation after backcrossing to *B. juncea* (Jørgensen 1999). Mustard greens is an

annual, introduced plant in Norway, located on waste ground in Southern Norway (Lid & Lid 2005). The species is now considered as established in Norway (sjekk med nyere versjon av Lid)

Black mustard (*B. nigra* (L.) W.D.J.Koch)

Reciprocal crossings under controlled conditions have demonstrated hybridization between *B. napus* and *B. nigra* (Bing et al. 1996). However, the hybridization frequency was low, being 0.01 % and 0.001 %, respectively. Hybridization between these species has not been observed in the field (Bing et al. 1996).

Hoary mustard (*B. adpressa* Boiss.)

B. adpressa can produce F₁ hybrids with *B. napus* (Lefol et al. 1996), but the introgression of *B. napus* genes into *B. adpressa* is not likely to be significant phenomenon because the hybrids have decreased fitness, reduced seed production, no viable seed and irregular chromosome numbers of the plants in each backcross generation with abortion of *B. napus* chromosomes frequently occurring (Darmency & Fleury 2000).

Wild radish (*Raphanus raphanistrum* ssp. *raphanistrum*)

Raphanus raphanistrum can hybridize with *B. napus*, but at a very low frequency (Gueritain et al. 2002). As reviewed in Devos (2009), seed dormancy of hybrids of *B. napus* and *R. raphanistrum* was within the range of their original parents and the hybrid plants had delayed seedling emerge, lower survival compared to both parents and produced less than two seeds per plant. Hybrids between these two species have reduced pollen viability (less than 1 %) (Warwick et al. 2003). The potential for hybridization between *B. napus* and *R. raphanistrum* under field conditions is extremely low, and, if it were to occur, the hybrids would have reduced survival and limited reproductive success.

Field mustard (*Sinapsis arvensis* L.)

Research on genetic exchange between *B. napus* and *S. arvensis*, both under natural conditions in the field and under controlled conditions, shows that the probability of hybridization between these species is very low (Bing et al. 1995; Moyes et al. 2002; Warwick et al. 2003). Hybridization has been reported in greenhouses (Moyes et al., 2002) and Daniels et al (2005) demonstrated hybrids at very low frequencies in the field. It has not been possible to detect genetic exchange between oilseed rape and field mustard in the field in a number of other studies (Bing et al. 1995; Chevre et al. 1996; Moyes et al. 2002; Warwick et al. 2003).

White mustard (*S. alba* L.)

No spontaneous crosses in the field have been reported between *B. napus* and *S. alba* (Daniels et al. 2005). Crossings under controlled conditions have demonstrated hybridization between these species, usually requiring embryo or ovule culture (ref. OECD 2011).

Common dog mustard (*Erucastrum gallicum* (Willd.) O.E.Schulz)

Genetic exchange between oilseed rape and common dog mustard has been the subject of few studies. There is one report on hybridization under controlled conditions, where only one hybrid plant was recorded (Lefol et al., 1997). Warwick et al. (2003) investigated hybridization between oilseed rape and glyphosate-resistant *E. gallicum* in commercial cultivation fields in Canada. Among a total of 22,000 seedlings that were examined for expression of herbicide resistance, no transgenic hybrids were detected. Common dog mustard has been introduced and become partially established in Norway.

Annual wall rocket (*Diplotaxis muralis*), **perennial wall rocket** (*D. tenuifolia* (L.) DC)

Hand crosses have been made in enclosed environments between *B. napus* and *Diplotaxis muralis* and *D. tenuifolia*. No field interspecific or intergeneric hybridshave been reported between and these species (ref. OECD 2011).

Several of the weed species in the *Brassica* complex readily form hybrids. Genetic exchange from oilseed rape to other incompatible species through a 'middle-species' (known as 'bridging'), has been

the subject of several studies (OGTG 2002). In most cases, *B. juncea* is considered as a possible intermediate host. *B. napus* x *B. juncea* hybrids are, however, relatively rare, have reduced fertility, and the seed have poor germination characteristics. Crossings between *B. juncea* and *B. nigra* are not fully compatible, and any crosses between a *B. napus* hybrid and *B. nigra* will thus have less compatibility. Most studies conclude that the risk of transfer of genes between these species via mustard greens is very small (OGTG 2002). *B. rapa* is also an unlikely 'intermediate host', as the F₁-hybrids are sterile or have low fertility, and there is no form of seed dormancy.

5.4 Potential interactions of the GM plant with target organisms

Interactions of oilseed rape MON 88302 with target organisms are not considered an issue by the VKM Panel on Genetically Modified Organisms, as there are no target organisms.

5.5 Potential interactions of the GM plant with non-target organisms (NTOs)

The scope of this application covers import and processing, and all uses as any other oilseed rape excluding cultivation. No deliberate release of viable plant material in the EU/EEA is expected and interactions of MON 88302 with the biotic environment will be very limited. Some accidental spillage of seed from MON 88302 may however occur along transportation routes, processing plants and storing facilities during import, handling, storage and processing. CP4 EPSPS is heat inactivated during processing for feed, and can also be inactivated in the digestive tract of animals. Given the low level of environmental exposure to MON 88302 to non-target organisms, the likelihood of adverse effects to NTO communities that perform in-field ecological functions and NTO communities outside the field from import of MON 88302 is negligible.

5.6 Potential impacts of the specific cultivation, management and harvesting techniques

Cultivation of MON 88302 in the EU is not included in the scope of the application EFSA/GMO/BE/2011/101. An assessment of the impacts of altered cultivation, management and harvesting techniques of MON 88302 is therefore not relevant given the scope of this application.

5.7 Potential interactions with the abiotic environment and biogeochemical cycles

The scope of the application covers import, processing, and food and feed use of MON 88302, and no deliberate release of viable plant material is expected in the EU/EEA and interactions of MON 88302 with the biotic environment will be very limited. The limited routes of exposure of soil micro-organisms to MON 88302 are through accidental seed release during transport and processing, and indirect exposure through manure or organic plant matter imported as a fertilizer or soil amendment from faeces of livestock fed MON 88302. The likelihood of exposure of soil micro-organism to active CP4 EPSPS protein via manure and faeces of livestock fed with processed or unprocessed seed of MON 88302 is negligible. CP4 EPSPS is heat inactivated during processing for feed, and will also be degraded via enzymatic activity in the gastro-intestinal tract of the animals. Given the low level of environmental exposure combined with a lack of hazard, the import, processing and food and feed uses of MON 88302 in the EU it is not likely to adversely impact soil micro-organisms that perform ecological functions in-field or in non-agricultural habitats, and therefore poses negligible environmental risk.

6 Post-Market Environmental Monitoring Plan

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. According to Annex VII, the objectives of an environmental monitoring plan are 1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the environmental risk assessment (ERA) are correct, and (2) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment.

Post-market environmental monitoring is composed of case-specific monitoring and general surveillance (EFSA 2011c). Case-specific monitoring is not obligatory, but may be required to verify assumptions and conclusions of the ERA, whereas general surveillance is mandatory, in order to take account of general or unspecific scientific uncertainty and any unanticipated adverse effects associated with the release and management of a GM plant. Due to different objectives between case-specific monitoring and general surveillance, their underlying concepts differ. Case-specific monitoring should enable the determination of whether and to what extent adverse effects anticipated in the environmental risk assessment occur during the commercial use of a GM plant, and thus to relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the ERA.

The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the ERA. In contrast to case-specific monitoring, the general status of the environment that is associated with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect any possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

6.1 Case-specific GM plant monitoring

When potential adverse effects or important gaps in scientific information or significant levels of critical uncertainty linked to the GM plant and its management have been identified in the environmental risk assessment, then case-specific monitoring should be carried out after placing on the market, in order to confirm assumptions made in the ERA and to further inform the ERA (EFSA 2011c). Case-specific monitoring should be targeted at assessment endpoints and environmental protection goals identified in the ERA conclusion as being at risk or where levels of critical uncertainty were identified in relation to potential risks associated with the GM plant. Monitoring of potentially adverse cumulative long-term or large-scale effects and the resolution of areas of critical uncertainty, identified in the ERA are important objectives of monitoring (EC 2002).

The scope of the application EFSA/GMO/BE/2011/101 is the authorisation of MON 88302 for import, processing and the use of food and feed produced from MON 88302 in the EU under Regulation (EC) No. 1829/2003. The scope of the application does not include authorisation for the cultivation of MON 88302 seed products. The environmental risk assessment, conducted by the applicant, support a conclusion that the import, processing and all uses as any other oilseed rape, but excluding the cultivation of MON 88302 in the EU, represents negligible risk to human and animal health and the environment, and poses no greater risk than the import and processing of conventional oilseed rape. Because no immediate adverse risk effects are expected, the probability of long-term adverse effects is

also negligible. The applicant has therefore considered that there is no need for case-specific monitoring.

However, accidental spillage and loss of viable seeds of MON 88302 during transport, storage, handling in the environment and processing into derived products is likely to take place over time. Oilseed rape can establish feral populations outside cultivated areas (e.g. roadsides, railway ground, ports) and escaped populations of herbicide-tolerant oilseed rape have been reported along transportation routes, ports and close to processing plants in Japan, Canada and USA (Yoshimura et al. 2006; Knispel et al. 2008; Nishizawa et al. 2009; Schafer et al. 2011). Germination and establishment of volunteer MON 88302 plants may result in gene flow into cultivated varieties and feral populations of *Brassica napus* as well as into closely related wild relatives (Knispel et al. 2008; Schafer et al. 2011). Furthermore GM-oilseed plants may cause potential problems for co-existence with conventional varieties in case of admixture which should be addressed. The Norwegian Panel on Genetically Modified Organisms is therefore of the opinion that the applicant should be requested to provide a case-specific monitoring plan covering spillage or loss of viable seed of MON 88302 oilseed rape during transport, storage and processing and use.

6.2 General surveillance for unanticipated adverse effects

According to the principles and objectives outlined in Annex VII of Directive 2001/18/EC, the objectives of general surveillance is to detect any unanticipated adverse effects on protected and valued entities of the environment, including biodiversity and ecosystem services (EFSA 2011c).

In the context of the intended uses of MON 88302, exposure to the environment will be limited to unintended release of rape seed, which could occur e.g. via losses during loading/unloading of viable commodity including MON 88302 destined for processing into animal feed or human food products.

The applicant proposed to conduct general surveillance for oilseed rape MON 88302 throughout the period of validity of the authorisation. According to the technical dossier from the applicant, the general surveillance will take into consideration, and be proportionate to, the extent of imports of MON 88302 and use thereof in the EU Member States. In order to increase the possibility of detecting any unanticipated adverse effects, a monitoring system will be used, which involves the authorisation holder and operators handling and using viable MON 88302. The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable seeds.

The applicant proposed to build its general surveillance on the following approaches; 1) Procedure(s) from the food/feed business operators based on the Hazard Analysis of Critical Control Point (HACCP) principles, 2) review of scientific information provided by existing monitoring network, 3) the monitoring and review of ongoing research and development, as well as scientific literature.

The scope of the monitoring plan provided by the applicant is in line with the intended uses for the event MON 88302.

The applicant will submit an annual monitoring report covering results of the general surveillance in accordance with the conditions of the authorisation. The report will contain information of any unanticipated adverse effects that have arisen from handling and use of viable MON 88302. According to the monitoring plan, the report will include a scientific evaluation of the confirmed adverse effect, a conclusion of the safety of MON 88302 and, as appropriate, the measures that were taken to ensure the safety of human and animal health or the environment.

7 Data gaps

- Routes of import, transport and processing of oilseed rape seeds in Norwegian environments, and quantitative considerations of the potential of spillage.
- Established whether feral populations of oilseed rape are short-lived or have a more permanent nature. Since the places where most substantial losses occur are most likely to show the first initial populations, particularly these places should be identified and studied.
- The presence, number and viability of rape seeds in the meal and cake from the crushing process and in the waste from cleaning operations.

8 Comments to the EFSA GMO Extranet - application EFSA/GMO/BE/2011/101

D. 12.02 Case-specific GM plant monitoring

The post-market environmental plan, submitted with the application EFSA/GMO/BE/2011/101, does not include a case-specific monitoring of MON 88302 oilseed rape. However, accidental spillage and loss of viable seeds of MON 88302 during transport, storage, handling in the environment and processing into derived products is likely to take place over time. Oilseed rape can establish feral populations outside cultivated areas (e.g. roadsides, railway ground, ports) and escaped populations of herbicide-tolerant oilseed rape have been reported along transportation routes, ports and close to processing plants in Japan, Canada and USA (Yoshimura et al. 2006; Knispel et al. 2008; Nishizawa et al. 2009; Schafer et al. 2011). Germination and establishment of volunteer MON 88302 plants may result in gene flow into cultivated varieties and feral populations of *Brassica napus* as well as into closely related wild relatives (Knispel et al. 2008; Schafer et al. 2011). Furthermore GM-oilseed plants may cause potential problems for co-existence with conventional varieties in case of admixture which should be addressed. The Norwegian Panel on genetically Modified Organisms is therefore of the opinion that the applicant should be requested to provide a case-specific monitoring plan covering spillage or loss of MON 88302 oilseed rape during transport, storage and processing.

Preliminary assessment based on available data

Molecular characterisation

The VKM Panel on Genetically Modified Organisms finds that the descriptions of the insert and inheritance have been sufficiently described. We also find it justified that there is only one major T-DNA insert in MON88302 and that no major section of the T-DNA plasmid backbone is inserted.

Comparative assessment

Based on results from comparative analyses of data from field trials located at representative sites and environments in the US, Canada and Chile, it is concluded that oilseed rape MON 88302 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of the herbicide tolerance conferred by the CP4 EPSPS protein. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of MON 88302 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glyphosate herbicide do not alter the phenotypic and agronomic characteristics of MON 88302 compared to conventional oilseed rape.

Evaluations of environmental interactions between genetically modified oilseed rape MON 88302 and the biotic and abiotic environment, and studies of seed dormancy, seed germination, pollen morphology and viability indicates no unintended effects of the introduced trait on these characteristics in MON 88302 oilseed rape.

Environmental risk

Considering the scope of the application EFSA/GMO/BE/2011/101, excluding cultivation purposes, the environmental risk assessment is limited to exposure through accidental spillage of viable seeds of MON 88302 into the environment during transportation, storage, handling, processing and use of derived products.

Oilseed rape is mainly a self-pollinating species, but has entomophilous flowers capable of both self- and cross-pollinating. Normally the level of outcrossing is about 30 %, but outcrossing frequencies up to 55 % are reported.

Several plant species related to oilseed rape that are either cultivated, occurs as weeds of cultivated and disturbed lands, or grow outside cultivation areas to which gene introgression from oilseed rape could be of concern. These are found both in the *Brassica* species complex and in related genera. A series of controlled crosses between oilseed rape and related taxa have been reported in the scientific literature. Because of a mismatch in the chromosome numbers most hybrids have a severely reduced fertility. Exceptions are hybrids obtained from crosses between oilseed rape and wild turnip (*B. rapa* ssp. *campestris*) and to a lesser extent, mustard greens (*B. juncea*), where spontaneously hybridising and transgene introgression under field conditions have been confirmed. Wild turnip is native to Norway and a common weed in arable lowlands.

There is no evidence that the herbicide tolerant trait results in enhanced fitness, persistence or invasiveness of oilseed rape MON 88302, or hybridizing wild relatives, compared to conventional oilseed rape varieties, unless the plants are exposed to glyphosate-containing herbicides.

However, accidental spillage and loss of viable seeds of MON 88302 during transport, storage, handling in the environment and processing into derived products is likely to take place over time, and the establishment of small populations of oilseed rape MON 88302 on locations where glyphosate is frequently applied to control weeds e.g. on railway tracks, cannot be excluded. Feral oilseed rape MON 88302 arising from spilled seed could theoretically pollinate conventional crop plants if the escaped populations are immediately adjacent to field crops, and shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops. However, both the occurrence of feral

oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape populations to wild populations are likely to be low in an import scenario. Apart from the glyphosate tolerance trait, the resulting progeny will not possess a higher fitness and will not be different from progeny arising from cross-fertilisation with conventional oilseed rape varieties.

The VKM GMO Panel concludes that this route of gene flow would not introduce significant numbers of transgenic plants into agricultural areas or result in any environmental consequences in Norway.

The environmental risk assessment will be completed and finalized by the VKM Panel on Genetically Modified Organisms when requested additional information from the applicant is available.

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Appendix 1

Table1. Phenological growth stages and BBCH-identification keys of oilseed rape (Weber & Bleiholder 1990; Lancashire et al. 1991)

Code	Description
Principal growth stage 0: Germination	
00	Dry seed
01	Beginning of seed imbibition
03	Seed imbibition complete
05	Radicle emerged from seed
07	Hypocotyl with cotyledons emerged from seed
09	Emergence: cotyledons emerge through soil surface
Principal growth stage 1: Leaf development	
10	Cotyledons completely unfolded
11	First leaf unfolded
12	2 leaves unfolded
1.	Stages continuous till.....
19	9 or more leaves unfolded
Principal growth stage 2: Formation of side shoots	
20	No side shoots
22	2 side shoots detectable
2.	Stages continuous till.....
29	End of side shoot development: 9 or more side shoots detectable
Principal growth stage 3: Stem elongation	
30	Beginning of stem elongation: no internodes (“rosette”)
31	1 visibly extended internode
32	2 visibly extended internodes
3.	Stages continuous till ...
39	9 or more visibly extended internodes
Principal growth stage 5: Inflorescence emergence	
50	Flower buds present, still enclosed by leaves
51	Flower buds visible from above (“green bud”)
52	Flower buds free, level with the youngest leaves
55	Individual flower buds (main inflorescence) visible but still closed
59	First petal visible, flower buds still closed («yellow bud»)
Principal growth stage 6: Flowering	
60	First flowers open
61	10% of flowers on main raceme open, main raceme elongating
62	20% of flowers on main raceme open
65	Full flowering: 50 % flowers on main raceme open, older petals failing
67	Flowering declining: majority of petals fallen
69	End of flowering

Principal growth stage 7: Development of fruit	
71	10 % of pods have reached final size
7.	xx % of pods have reached final size
78	80 % of pods have reached final size
79	Nearly all pods have reached final size
Principal growth stage 8: Ripening	
80	Beginning of ripening: seed green, filling pod cavity
81	10 % of pods ripe, seeds dark and hard
82	20 % of pods ripe, seeds dark and hard
8.	xx % of pods ripe, seeds dark and hard
88	80 % of pods ripe, seeds dark and hard
89	Fully ripe: nearly all pods ripe, seeds dark and hard
Principal growth stage 9: Senescence	
97	Plant dead and dry
99	Harvested product

Processing of rapeseed (OECD 2009)

Oilseed rape seed is traditionally crushed and solvent extracted in order to separate the oil from the meal. The process usually includes seed cleaning, seed pre-conditioning and flaking, seed cooking/conditioning, pressing the flake to mechanically remove a portion of the oil, solvent extraction of the press-cake to remove the remainder of the oil, oil and meal desolventizing, degumming and refining of the oil, and toasting of the meal (OECD 2009). The main steps of the process are schematised in Figure 1.

1. Seed cleaning

The seed is cleaned to remove plant stalks, grain seeds and other materials from the bulk of the seed. Aspiration, indent cleaning, sieving, or some combination of these is used in the cleaning process. Dehulling of the seed is, at present, not a commercial process.

2. Seed pre-conditioning and flaking

Many crushing plants in colder climates preheat the seed to approximately 35°C through grain dryers in order to prevent shattering which may occur when cold seed from storage enters the flaking unit (Unger, 1990). The cleaned seed is first flaked by roller mills set for a narrow clearance to physically rupture the seed coat. The objective here is to rupture as many cell walls as possible without damaging the quality of the oil. The thickness of the flake is important, with an optimum of between 0.3 to 0.38 mm. Flakes thinner than 0.2 mm are very fragile while flakes thicker than 0.4 mm result in lower oil yield.

3. Seed cooking/conditioning

Flakes are cooked/conditioned by passing them through a series of steam-heated drum or stack-type cookers. Cooking serves to thermally rupture oil cells which have survived flaking, reduce oil viscosity and thereby promote coalescing of oil droplets, increase the diffusion rate of prepared oil cake, and denature hydrolytic enzymes. Cooking also adjusts the moisture of the flakes, which is important in the success of subsequent pre-pressing operations. At the start of cooking, the temperature is rapidly increased to 80-90°C. The rapid heating serves to inactivate the myrosinase enzyme present in canola. This enzyme can hydrolyse the small amounts of glucosinolates present in canola and will produce undesirable breakdown products which affect both oil and meal quality. The cooking cycle usually lasts 15 to 20 minutes and the temperatures usually range between 80 and 105°C, with an optimum of about 88°C. In some countries, especially China, cooking temperatures of up to 120°C have been traditionally used when processing high glucosinolate rapeseed to volatilize some of the sulphur compounds which can cause odours in the oil. However, these high temperatures can negatively affect meal protein quality.

4. Pressing

The cooked canola seed flakes are then pressed in a series of low pressure continuous screw presses or expellers. This action removes most of the oil while avoiding excessive pressure and temperature. The objective of pressing is to reduce the oil content of the seed from about 42% to 16-20%, making the solvent extraction process more economical and efficient, while producing acceptable quality presscake.

5. Solvent extraction

Since the pressing is not able to remove all of the oil from the canola seed, the presscake is solvent extracted to remove the remaining oil. The cake from the expellers, containing between 14 and 20% oil, is sometimes broken into uniform pieces prior to solvent extraction. In solvent extraction, hexane specially refined for use in the vegetable oil industry is used. After a series of extractions, the marc (hexane saturated meal) that leaves the solvent extractor, contains less than 1% oil.

6. Desolventizing of oil and meal

The micella and meal are “stripped” of solvent, to recover solvent-free oil and meal. The micella containing the oil is desolventised using evaporator equipment. The solvent is removed from the marc in a desolventiser-toaster. This is done in a series of compartments or kettles within the desolventiser, often by injection of live steam, followed by final stripping and drying at a temperature of 103-107°C. The final, solvent-free meal contains about 1% oil and 8 to 10% moisture.

7. Degumming of oil

The “crude” oil from the two extraction stages is usually blended and then degummed before being stored for sale or further processing. Degumming removes phosphatides co-extracted with the oil, which tend to separate from the oil as sludge during storage. The phosphatide content of crude oil varies, but is usually in the order of 1.25%, or measured as phosphorus, 500 ppm. Two degumming methods are in use: (a) using water to precipitate phosphatides and; (b) using an acid such as citric, malic, or phosphoric and water (super-degumming).

8. Alkali and physical refining of oil

Degummed oil is further purified in a process of refining. One of two methods are used, namely, alkali refining, especially with water degummed oil, and physical refining with acid-water degummed oil. Alkali refining is the most common process used, even with acid-water degummed oil. Physical refining is a relatively new development. It requires well-degummed oil of moderate chlorophyll and free fatty acid content, but it is then very economical. Alkali refining reduces soap, free fatty acid, phosphorus levels. The further removal of free fatty acids is done by steam distillation in a deodorizer. This simultaneously deodorizes the oil. Because deodorization is the last process normally carried out on edible oils, this step may be delayed until other processes, such as hydrogenation of the oil, have been done. Alkali-refined oil contains chlorophylloid compounds which give the oil a green colour, and catalyse oil oxidation. These compounds are removed by adsorptive bleaching with acid-activated clays.

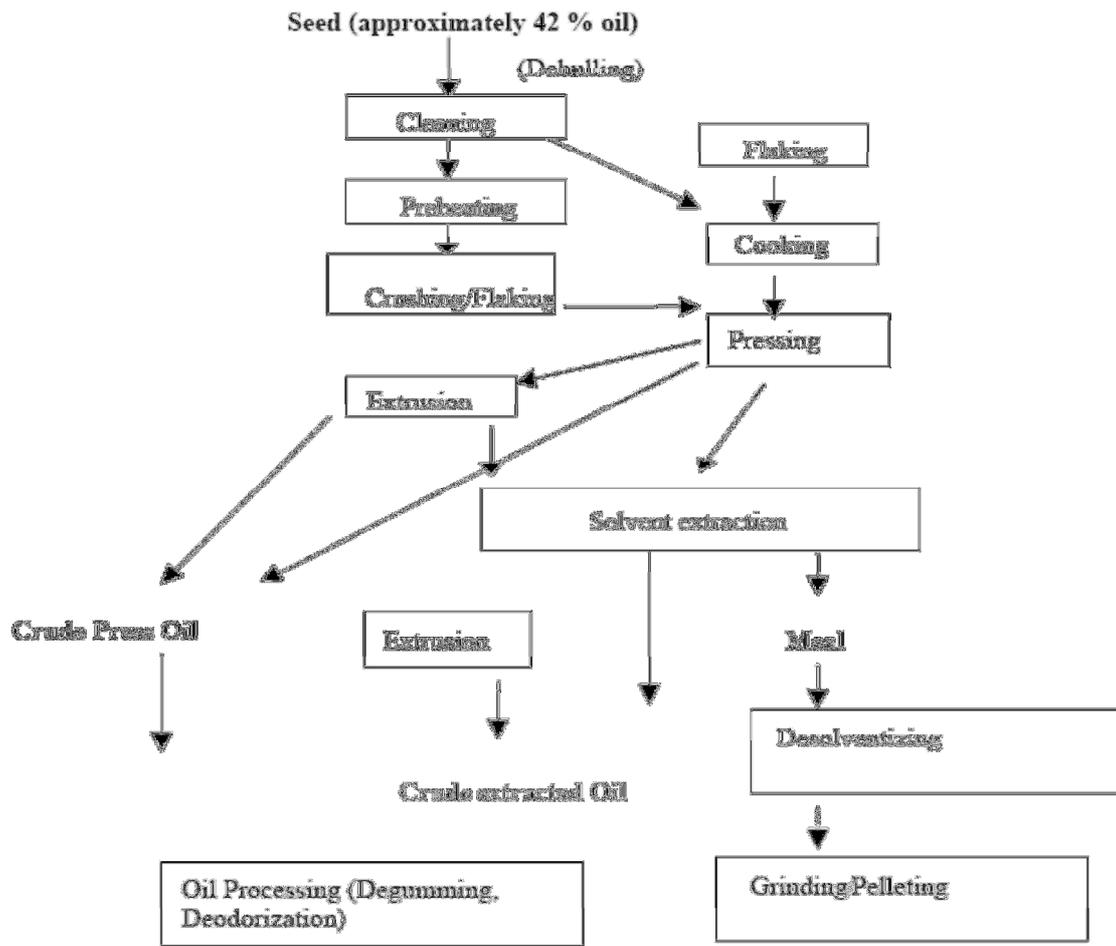


Figure 1 Schematic illustration of the processing of low erucic acid rapeseed meal and low erucic acid rapeseed oil (OECD 2001).

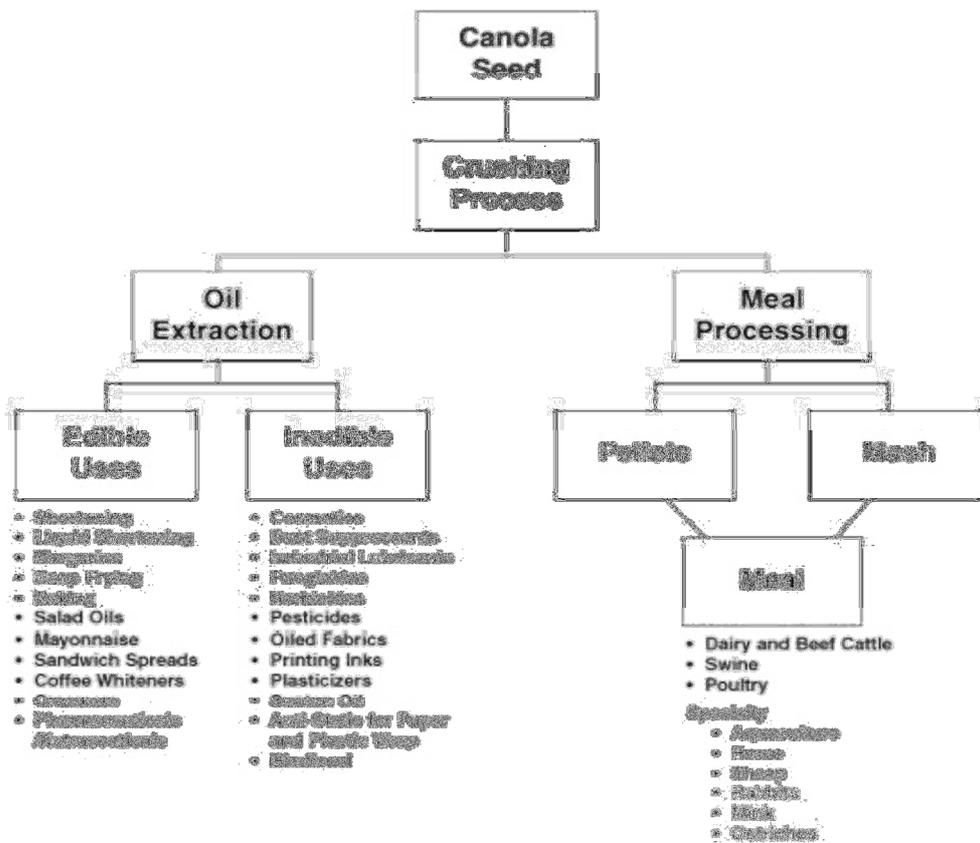


Figure 2 Areas of application and products from processing of rapeseed (Canola Council of Canada 2005).

Tabell 3. Oversikt over registreringer av agronomiske og fenotypiske karakterer og mottagelighet for biotiske og abiotiske stressfaktorer i feltforsøk i Canada og Europa i perioden 1992-1996.

Parameters observed		Field Trial (Year)				
		Canada (1992-1994)	Belgium (1993)	Belgium (1994)	France (1994)	UK (1994)
Agronomic and Phenotypic Characteristics						
Germination/Emergence	Germination rate/Emergence	Yes	Yes	Yes		
Vegetative growth	Stand count		Yes	Yes	Yes	
	Plant vigor	Yes	Yes	Yes		
	Color		Yes			
	Height		Yes	Yes	Yes	
	Developmental stage (Maturity)	Yes	Yes	Yes	Yes	
Reproductive growth	Flowering Period	Yes	Yes	Yes	Yes	Yes
	Pollen produced/viability (indirect through harvest)		Yes	Yes		Yes
	Yield	Yes	Yes	Yes		Yes
Volunteer/Persistence	Volunteer/Persistence	Yes	Yes	Yes		
	Silique shattering and dispersal	Yes				
	Seed dormancy	Yes				

Table 6 List of parameters observed for the field trials conducted where agronomic, phenotypic and susceptibility to biotic and abiotic stressors were observed (continued)

Parameters observed		Field Trial (Year)				
		Canada (1992-1994)	Belgium (1993)	Belgium (1994)	France (1994)	UK (1994)
Plant interactions with insect, disease, and abiotic stressors						
Susceptibility to diseases	Mildew	Yes		Yes		
	Alternaria	Yes	Yes		Yes	
	Botrytis	Yes			Yes	
	Cylindrosporium				Yes	
	Phoma				Yes	
	Sclerotinia	Yes			Yes	
	Others	Yes			Yes	
Susceptibility to insects	Aphids			Yes	Yes	
	Pollen beetle (<i>Meligethes aeneus</i>)		Yes	Yes	Yes	Yes
	Flea beetle (<i>Phyllotetra Spp.</i>)	Yes			Yes	Yes
	Rape winter stem weevil (<i>Ceutorhynchus pictarisis</i>)				Yes	
	Rape stem weevil (<i>Ceutorhynchus napi</i>)				Yes	
	Others	Yes				
Susceptibility to abiotic stressors	Herbicides (other than glyphosate)		Yes	Yes		
	Insecticides		Yes	Yes		
	Fungicides		Yes	Yes		

Appendix 2

Potential for cross-pollination and introgression with other *Brassica* species

Wild turnip (*B. rapa* ssp. *campestris* (L.) A.R. Clapham)

A number of studies have shown that hybridization between *B. napus* and *B. rapa* ssp. *campestris* occurs spontaneously in the field (e.g., Jørgensen & Andersen 1994; Landbo et al. 1996; Mikkelsen et al. 1996; Jørgensen et al. 1996, 1998; Halfhill et al. 2004). Hybridization between these species can occur in both directions, but primarily arises with *B. rapa* ssp. *campestris* as the pollen donor. The hybridization frequency between these species is reported to range from 0 to 93 %, depending on experimental design, cultivar characteristics, and environmental conditions. Danish studies have shown that individual plants of *B. rapa* in crop fields with autumn oilseed rape produced an average of 265 hybrids per plant (i.e., 93 % F₁-hybrids) (Jørgensen et al. 1996). This is because *B. rapa* is an obligate out-crosser, and when isolated from other pollen sources due to experimental design there will be little competition for *B. napus* from other pollinators (Anon. 1999; Eastham & Sweet, 2002). When *B. rapa* and *B. napus* were grown at a 1:1 ratio, hybridization frequencies of 13 % and 9 % were observed, depending on whether *B. rapa* or *B. napus* was used as the parent plants. This illustrates that compatibility with pollen from *B. rapa* is higher than compatibility with *B. napus* pollen.

F₁-hybrids are triploid (2n = 29, AAC), sterile, or have reduced pollen fertility (Stace 1997; Warwick et al. 2003). The potential for dissemination to natural habitats will therefore be largely related to the introgression of transgenes into the weed population. Controlled experiments in the field or greenhouse (Jørgensen & Andersen 1994; Jørgensen et al. 1996; Mikkelsen et al. 1996) and experiments associated with commercial cultivation (Hansen et al. 2001; Warwick et al. 2003) have shown that backcrossing between F₁-hybrids and *B. rapa* ssp. *campestris* can occur spontaneously. A large number of backcrossed plants have also been shown to have high fertility. Snow et al. (1999) found that the BC₃-generation had a pollen fertility corresponding to 88-95 % and that the plants were as vigorous as pure *B. rapa* plants. Repeated backcrossing results in gradual loss of the C-chromosomes, with the exception of regions that are recombined into the A-genome (Johannessen 2004).

Extensive introgression has been reported from a mixed population of *B. napus* and *B. rapa* in organically farmed fields in Denmark, 11 years after conversion (Hansen et al. 2001). Of 102 plants analysed, only one individual was a first generation hybrid (F₁-hybrid), while almost half of the plants had specific genetic markers from both *B. napus* and *B. rapa*. Warwick et al. (2003) registered a hybridization frequency of up to 13.6 % between a weed population and cultivated oilseed rape plants in a commercial plantation in Canada. A later study by the same research group also demonstrated that transgenic hybrids have considerable potential to produce transgenic offspring through backcrossing (Halfhill et al. 2004). The frequency of backcrossing between *B. rapa* and transgenic hybrids with *Bt*-resistance was reported to be about 50 % in those cases where *B. rapa* was the pollen donor. If hybrid plants were the pollen source, backcrossing frequencies of 0.088 % and 0.060 %, respectively, were observed. After a generation of backcrossing between herbicide-resistant F₁-hybrids and *B. rapa* ssp. *campestris*, a large proportion of the offspring were found to be morphologically and cytologically identical to *B. rapa* ssp. *campestris*, and after repeated backcrossing to *B. rapa* around 10 % of BC₃-hybrids and BC₄-hybrids were reported to be resistant to herbicides (Metz et al. 1997).

The first report that documents the persistence and stable incorporation of transgenes from herbicide-resistant oilseed rape into *B. rapa* ssp. *campestris* in commercial cultivation fields was published in 2008 by Warwick et al. (Warwick et al. 2008). The fields where the research group demonstrated hybridization between glyphosate-tolerant *B. napus* and weed populations of *B. rapa* in Canada in 2001 were also monitored during the growing seasons of 2002, 2003, and 2005. Although the number of hybrids was dramatically reduced from 2002 to 2005, transgene persistence was confirmed in one of the two populations of *B. rapa* over a period of 6 years, despite the fact that the plants were not

exposed to selective pressures in the form of glyphosate treatment and reduced pollen fertility. This was demonstrated in both F₁-generations and backcrossed generations of the hybrid.

Turnip mustard is native to Norway. The species is a common weed in arable lowlands and is also widely distributed in the villages in the valleys and mountains in southern Norway and the most northerly counties (Lid & Lid 2005).

Mustard greens (leaf mustard) (*B. juncea* (L.) Czern.)

B. juncea and *B. napus* have a common set of chromosomes and are known to be sexually compatible. Hybrids have been produced by controlled crossings (Mikkelsen & Jørgensen 1997), and it is also known that the hybrids can form spontaneously under natural field conditions (Frello et al. 1995; Jørgensen et al. 1996; Liu et al. 2010. As reviewed in Devos (2009), in field plots with interplanted *B. napus* and *B. juncea* interspecific hybridization frequencies were low. In a Danish study, Jørgensen et al. (1996) reported a 3 % hybridization frequency from crossings with *B. napus* as a pollinator. Equivalent results have been reported from Canada (Bing et al. 1991; Eastham & Sweet 2002). Species hybridization can occur in both directions, but is most successful with *B. napus* as the pollen donor. The F₁-hybrid has low fertility (0 – 28 %), but expression of transgenes has been observed in the first generation after backcrossing to *B. juncea* (Jørgensen 1999).

Mustard greens is an annual, introduced plant in Norway, originating from Central and Eastern Asia. It is found in waste sites, particularly in Hedmark and Oppland, and also in some localities in the coastal regions from Østfold to Trøndelag (Lid & Lid 2005). It has recently been reported on several occasions and may now perhaps be considered as established in Norway.

Black mustard (*B. nigra* (L.) W.D.J.Koch)

Black mustard does not produced hybrids in field plots with inter-planted *B. napus* (Bing et al. 1996). Reciprocal crossings under controlled conditions have demonstrated hybridization between *B. napus* and *B. nigra* when embryo rescue was performed and only when *B. napus* was the female parent. (Bing et al. 1996). However, the hybridization frequency was low, being 0.01 % and 0.001 %, respectively. Reduced pollen fertility (0-1.9%) in the resulting hybrids (Kerlan et al. 1992) ensures that even if such a cross were to occur, reduced reproductive success makes introgression highly unlikely. The likelihood of gene flow from *B. napus* to *B. nigra* under field conditions is extremely low.

In Norway, black mustard is an introduced species and appears sporadically on waste sites and fallow land in the coastal areas from Østfold to Trøndelag (Lid & Lid 2005). The species has also been reported from some individual locations in inland regions of Norway.

Hoary mustard (*B. adpressa* Boiss.)

Hybridization between *B. napus* and *B. adpressa* occurs spontaneously in the field, primarily with hoary mustard as the pollen source (Lefol et al. 1996; Darmency & Fleury 2000). In one study in which *B. adpressa* and transgenic oilseed rape were planted in a ratio of 1:625, 1.5 % F₁-hybrids were registered (Lefol et al. 1996). In cases where sterile male oilseed rape was used as parent plants in a 1:1 ratio, a 70 % hybridization frequency was reported.

Darmency & Fleury (2000) observed an average hybridization frequency of 0.6 hybrids per plant in crossings in which *B. napa* was the pollinator. *B. napus* x *B. adpressa* hybrids have lower fertility than the parent plants. Backcrossing to *B. adpressa* through 5 generations did not result in the production of viable offspring (Darmency & Fleury 2000).

Hoary mustard was first recorded in Norway in the 1920s and is now established in some locations in the coastal areas from Østfold to Trøndelag (Lid & Lid 2005). The species is probably spreading.

Wild radish (*Raphanus raphanistrum* ssp. *raphanistrum*)

Research from France, Australia, and Canada has shown that hybridization between *B. napus* and *R. rapanistrum* can occur spontaneously in the field, but that the rate is very low (Eber et al. 1994;

Chèvre et al. 1997, 1998, 2000; Rieger et al. 2001; Warwick et al. 2003). Depending on genotype, Chèvre et al. (2000) have suggested hybridization frequencies of between 10^{-7} and 10^{-5} . Corresponding estimates have been reported from field trials in Australia and Canada (Rieger et al. 2001; Warwick et al. 2003). The studies show reciprocal differences in crossings between these species. *B. napus* x *R. raphanistrum*-hybrids have chromosome numbers $2n = 37$ (RrRrAC), and have a highly unstable genomic structure and low pollen vitality. In crossings where male sterile oilseed rape served as parent plants, each oilseed rape plant produced, on average, 45 hybrid seeds (Darmency et al. 1998). When these F₁-hybrids were grown in mixtures with wild radish, it was found that each hybrid produced less than one offspring. However, the fertility was improved in later backcrossings to the weed species. Stable integration of genetic material from *B. napus* into the genome of *R. raphanistrum* has not been observed (Jørgensen 1999; Eastham & Sweet 2002).

Wild radish is an introduced and established weed in Norway (Lid & Lid 2005). The species is fairly common in fields and on fallow land north to the county Nord Trøndelag.

Field mustard (*Sinapsis arvensis* L.)

Research on genetic exchange between *B. napus* and *S. arvensis*, both under natural conditions in the field and under controlled conditions, shows that the probability of hybridization between these species is very low (Bing et al. 1995; Moyes et al. 2002; Warwick et al. 2003). Hybridization has been reported in greenhouses (Moyes et al., 2002) and Daniels et al (2005) demonstrated hybrids at very low frequencies in the field. It has not been possible to detect genetic exchange between oilseed rape and field mustard in the field in a number of other studies (Bing et al. 1995; Chevre et al. 1996; Moyes et al. 2002; Warwick et al. 2003).

Field mustard is an introduced and established weed that is found in fields, roadsides and waste ground in Norway (Lid & Lid 2005). The species has been in decline in recent years.

Common dog mustard (*Erucastrum gallicum* (Willd.) O.E.Schulz)

Genetic exchange between oilseed rape and common dog mustard has been the subject of few studies. There is one report on hybridization under controlled conditions, where only one hybrid plant was recorded (Lefol et al., 1997). Warwick et al. (2003) investigated hybridization between oilseed rape and glyphosate-resistant *E. gallicum* in commercial cultivation fields in Canada. Among a total of 22,000 seedlings that were examined for expression of herbicide resistance, no transgenic hybrids were detected. Common dog mustard has been introduced and become partially established in Norway. The species is found in certain locations along the coast between Østfold and Trøndelag (Lid & Lid 2005).

Several of the weed species in the *Brassica* complex readily form hybrids. Genetic exchange from oilseed rape to other incompatible species through a 'middle-species' (known as 'bridging'), has been the subject of several studies (OGTG 2002). In most cases, *B. juncea* is considered as a possible intermediate host. *B. napus* x *B. juncea* hybrids are, however, relatively rare, have reduced fertility, and the seed have poor germination characteristics. Crossings between *B. juncea* and *B. nigra* are not fully compatible, and any crosses between a *B. napus* hybrid and *B. nigra* will thus have less compatibility. Most studies conclude that the risk of transfer of genes between these species via mustard greens is very small (OGTG 2002). *B. rapa* is also an unlikely 'intermediate host', as the F₁-hybrids are sterile or have low fertility, and there is no form of seed dormancy.