Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Environmental risk assessment of glufosinate-tolerant genetically modified oilseed rape MS8, RF3 and MS8 x RF3 for import, processing and feed uses under Directive 2001/18/EC (Notification C/BE/96/01)

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

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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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Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Directorate for Nature Management to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Directorate requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The genetically modified, glufosinate-tolerant oilseed rape lines MS8, RF3 and MS8 x RF3 (Notification C/BE/96/01) are approved under Directive 2001/18/EC for import and processing for feed and industrial purposes since 26 March 2007 (Commission Decision 2007/232/EC). In addition, processed oil from genetically modified oilseed rape derived from MS8, RF3 and MS8 x RF3 were notified as existing food according to Art. 5 of Regulation (EC) No 258/97 on novel foods and novel food ingredients in November 1999. Existing feed and feed products containing, consisting of or produced from MS8, RF3 and MS8 x RF3 were notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003 and were placed on the market in January 2000.

An application for renewal of the authorisation for continued marketing of existing food, food ingredients and feed materials produced from MS8, RF3 and MS8 x RF3 was submitted within the framework of Regulation (EC) No 1829/2003 in June 2007 (EFSA/GMO/RX/MS8/RF3). In addition, an application covering food containing or consisting of, and food produced from or containing ingredients produced from oilseed rape MS8, RF3 and MS8 x RF3 (with the exception of processed oil) was delivered by Bayer CropScience in June 2010 (EFSA/GMO/BE/2010/81).

The VKM GMO Panel has previously issued a scientific opinion related to the notification C/BE/96/01 for the placing on the market of the oilseed rape lines for import, processing and feed uses (VKM 2008). The health and environmental risk assessment was commissioned by the Norwegian Directorate for Nature Management in connection with the national finalisation of the procedure of the notification C/BE/96/01 in 2008. Due to the publication of updated guidelines for environmental risk assessments of genetically modified plants and new scientific literature, the VKM GMO Panel has decided to deliver an updated environmental risk assessment of oilseed rape MS8, RF3 and MS8 x RF3.

A scientific opinion on an application for the placing on the market of MS8/RF3 for food containing or consisting of, and food produced from or containing ingredients produced from MS8/RF3 (with the exception of processed oil) (EFSA/GMO/BE/2010/81) have also been submitted by the VKM GMO Panel (VKM 2012).

The environmental risk assessment of the oilseed rape MS8, RF3 and MS8 x RF3 is based on information provided by the notifier in the applications EFSA/GMO/RX/MS8/RF3, EFSA/GMO/BE/2010/8, the notification C/BE/96/01, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

The VKM GMO Panel has evaluated MS8, RF3 and MS8 x RF3 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in 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 MS8, RF3 and MS8 x RF3 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 emphasised 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 genetically modified oilseed rape lines MS8 and RF3 were developed to provide a pollination control system for production of F_1-hybrid seeds (MS8 x RF3). Oilseed rape is a crop capable of undergoing both self-pollination (70%) as well as cross-pollination (30%). Therefore a system to ensure only cross-pollination is required for producing hybrids from two distinct parents. As a result of hybrid vigor cross-pollinated plants produce higher yield as compared to self-pollinating rape.

The hybrid system is achieved using a pollination control system by insertion and expression of *barnase* and *barstar* genes derived from the soil bacterium *Bacillus amyloliquefaciens* into two separate transgenic oilseed rape lines. The *barnase* gene in the male sterile line MS8 encode a ribonuclease peptide (RNase), expressed in the tapetum cells during anther development. The RNase effect RNA levels, disrupting normal cell function, arresting early anther development, and results in the lack of viable pollen and male sterility.

The fertility restoration line RF3 contains a *barstar* gene, coding for a ribonuclease inhibitor (Barstar peptide) expressed only in the tapetum cells of the pollen during anther development. The peptide specifically inhibits the Barnase RNase expressed by the MS8 line. The RNase and the ribonuclease inhibitor form a stable one-to-one complex, in which the RNase is inactivated. As a result, when pollen from the receptor line RF3 is crossed to the male sterile line MS8, the MS8 x RF3 progeny expresses the RNase inhibitor in the tapetum cells of the anthers allowing hybrid plants to develop normal anthers and restore fertility.

The *barnase* and *barstar* genes in MS8 and RF3 are each linked with the *bar* gene from *Streptomyces hygroscopus.* The *bar* gene is driven by a plant promoter that is active in all green tissues of the plant, and encodes the enzyme phosphinothricin acetyltransferase (PAT). The PAT enzyme inactivates phosphinothricin (PPT), the active constituent of the non-selective herbicide glufosinate-ammonium. The bar gen were transferred to the oilseed rape plants as markers both for use during *in vitro* selection and as a breeding selection tool in seed production.

Molecular characterisation

The oilseed rape hybrid MS8xRF3 is produced by conventional crossing. The parental lines MS8 and RF3 are well described in the documentation provided by the applicant, and a number of publications support their data. It seems likely that MS8 contains a complete copy of the desired T-DNA construct including the *bar* and *barnase* genes. Likewise, the event RF3 is likely to contain complete copies of the *bar* and *barstar* genes in addition to a second incomplete non-functional copy of the *bar*-gene. The inserts in the single events are preserved in the hybrid MS8xRF3, and the desired traits are stably inherited over generations.

Oilseed rape MS8, RF3 and MS8xRF3 and the physical, chemical and functional characteristics of the newly expressed proteins have previously been evaluated by the VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2008, 2012). The GMO Panel finds the characterisation of the physical, chemical and functional properties of the recombinant inserts in the oilseed rape

transformation events MS8, RF3 and MS8xRF3 to be satisfactory. The GMO Panel has not identified any novel risks associated with the modified plants based on the molecular characterisation of the inserts.

Comparative assessment

Based on results from comparative analyses of data from field trials located at representative sites and environments in Europe and Canada, it is concluded that oilseed rape MS8, RF3 and MS8 x RF3 is agronomically and phenotypically equivalent to the conventional counterpart, except for the newly expressed barnase, barstar and PAT proteins.

The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glufosinate herbicide do not alter the phenotypic and agronomic characteristics of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape varieties.

Environmental risk

Considering the scope of the notification C/BE/96/01, excluding cultivation purposes, the environmental risk assessment is limited to exposure through accidental spillage of viable seeds of MS8, RF3 and MS8 x RF3 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.

Accidental spillage and loss of viable seeds of MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3 cannot be excluded. Feral oilseed rape MS8, RF3 and MS8 x RF3 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 in Norway.

There is no evidence that the herbicide tolerant trait results in enhanced fitness, persistence or invasiveness of oilseed rape MS8, RF3 and MS8 x RF3, or hybridizing wild relatives, compared to conventional oilseed rape varieties, unless the plants are exposed to herbicides with the active substance glufosinate ammonium. Apart from the glufosinate 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.

Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 for reasons of reproductive toxicity.

Overall conclusion
The VKM GMO Panel concludes that oilseed rape MS8, RF3 and MS8xRF3 are unlikely to have any adverse effect on the environment in Norway in the context of its intended usage.

Keywords

GMO, Oilseed rape, *Brassica napus* ssp. *oleifera* (DC.) Metzg., genetically modified oilseed rape MS8, RF3, MS8 x RF3, hybrid, C/BE/96/01, glufosinate-tolerant, *bar, barnase, barstar,* PAT protein, environmental risk assessment, import, processing, Directive 2001/18/EC

Norsk sammendrag

I forbindelse med forberedelse til implementering av EU-forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Direktoratet for naturforvalting (DN) om å utarbeide endelige miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent i EU under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. DN har bedt VKM om endelige risikovurderinger for de EU-godkjente søknadene hvor VKM ikke har avgitt endelig miljørisikovurdering. I tillegg har DN bedt VKM vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige miljørisikovurderingene som VKM tidligere har levert.

De genmodifiserte oljerapslinjene MS8, RF3 and MS8 x RF3 (Notifisering C/BE/96/01) ble godkjent til import og prosessering til fôr og industrielle formål under EU-direktiv 2001/18 26. mars 2007 (Kommisjonsbeslutning 2007/232/EC). I tillegg ble prosesserte oljer fra MS8 og RF3, og MS8xRF3 godkjent under den forenklede prosedyren i Novel Foodsforordningen (EF) Nr. 258/97 i november 1999. Rapslinjene er videre notifisert som eksisterende produkt under forordning 1829/2003/EF, artikkel 8 og 20, til bruk som mel i fôrvarer og som næringsmiddel i form av prosessert olje.

I 2007 leverte Bayer CropScience en søknad om fornyet godkjenning av rapslinjene som prosesserte næringsmidler, næringsmiddelingredienser og fôrmidler under EU-forordning 1829/2003 (EFSA/GMO/RX/MS/RF3). Videre ble det i 2010 fremmet en søknad om godkjenning av MS8/RF3 som mat (dvs. næringsmidler som inneholder eller består av de genmodifiserte plantene og næringsmidler som er produsert fra eller inneholder ingredienser fra de genmodifiserte plantene) (EFSA/GMO/BE/2010/81). Søknaden gjelder imidlertid ikke prosessert olje og er fremmet for å komplettere allerede godkjente bruksområder for MS8/RF3. I henhold til søker var bakgrunnen for søknaden å ivareta/dekke opp for utilsiktet innblanding av sporforurensinger av MS8/RF3 i matkjeden.

Rapslinjene MS8/RF3 (C/BE/96/01) har tidligere vært vurdert av VKM med hensyn på helse- og miljøeffekter i forbindelse med vurdering av markedsadgang i Norge (VKM 2008). Etablering av nye, reviderte retningslinjer for miljørisikovurdering av genmodifiserte planter og publisering av ny vitenskapelig litteratur har medført at VKM har valgt å utarbeide en ny, oppdatert miljørisikovurdering av MS8, RF3 og MS8xRF3. VKMs faggruppe for GMO har også vurdert rapslinjene i forbindelse med EFSAs offentlige høring av søknad EFSA/GMO/BE/2010/81 (VKM 2012).

Risikovurderingen av de genmodifiserte rapslinjene er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSAs 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 EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler (EFSA 2006, 2010, 2011a,b,c).

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

Foreldrelinjene MS8 og RF3 er utviklet for å sikre kontroll med pollinering ved produksjon av F_1-hybridfrø (MS8xRF3). Oljeraps er i overveiende grad en selvfertil art, med omlag 70 %

selvpollinering og for å produsere F_1-hybrider er det derfor nødvendig å forhindre plantenes selvpollinering.

Hybridiseringssystemet "SeedLink" består av to transgene foreldrelinjer, en hannsteril linje MS8, samt RF3, en linje som gjenoppretter fertiliteten og som brukes som hannplante. MS8-plantene, som benyttes som morplanter, inneholder *barnase*-genet isolert fra jordbakterien *Bacillus amyloliquefaciens* under kontroll av den pollenspesifikke *PTA29*-promotoren. *Barnase*-genet koder for et ekstracellulært ribonuklease-enzym (RNase), som uttrykkes i tapetcellene i pollensekkene under utvikling av pollenknappene, og som bryter ned RNA i pollen. MS8-linjen produserer derfor ikke levedyktig pollen og kan ikke selvpollinere. RF3-linjen har fått overført det bakterielle genet *barstar* fra *B. amyloliquefaciens*, under kontroll av samme promotor (*PTA29*). Genet koder for en ribonukleaseinhibitor som uttrykkes i pollenknappenes tapetceller og som binder seg til, og inaktiverer barnaseproteinet. Ved konvensjonelle kryssinger med den hannsterile linjen MS8 vil derfor fertiliteten bli gjenopprettet, og F_1-hybridplantene vil produsere fertilt pollen. Begge foreldrelinjene har fått innsatt et *bar*-gen fra jordbakterien *Streptomyces hygroscopius.* Genet koder for enzymet fosfinotricin acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium, virkestoffet i fosfinotricin-herbicider (preparat Finale mfl.). Rapslinjene MS8 og RF3 inneholder ingen markørgener for antibiotikaresistens.

Molekylær karakterisering
VKMs faggruppe for GMO vurderer karakteriseringen av de rekombinante DNA-innskuddene i MS8 og RF3 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 det rekombinante innskuddet i rapslinjene. Dette er i overenstemmelse med faggruppens tidligere vurderinger av rapslinjene (VKM 2008, 2012).

Komparative analyser
Feltforsøk i Canada og Europa indikerer agronomisk og fenotypisk ekvivalens mellom de transgene rapslinjene MS8, RF3 and MS8 x RF3 og umodifisert kontroll.

Miljørisiko
Notifisering C/BE/96/01 omfatter import, prosessering og bruk av de genmodifiserte oljerapslinjene MS8, RF3 og MS8 x RF3 til fôr. Miljørisikovurderingen av MS8, RF3 og MS8 x RF3 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. Det er vist at oljeraps kan danne spontane hybrider med åkerkål (*B. rapa* ssp. *campestris*), et vanlig åkerugras i hele Sør-Norge. Det er også rapport om spontan hybridisering i felt med sareptasennep (*B. juncea*), men hybridiseringsfrekvensene er svært lave og utbredelsen av denne arten er marginal i Norge.

Det er ingen indikasjoner på økt risiko for spredning, overlevelse og etablering av rasplinjen MS8, RF3 and MS8 x RF3 som naturaliserte populasjoner utenfor dyrkingsområder eller for utvikling av ugraspopulasjoner sammenlignet med ikke-genmodifisert raps. Herbicidtoleranse er selektivt nøytralt i naturlige habitater, og kan bare betraktes å ha økt fitness hvor og når herbicider med glufosinat-ammonium anvendes. Glufosinat-ammonium har helseklassifisering for både akutte og kroniske skadevirkninger på pattedyr inkludert mennesker, og ble trukket fra det norske markedet i 2008. I EU er virkestoffet under utfasing og er kun tillatt benyttet fram til 2017.

Ferale rapsplanter med opphav fra frøspill ved transport, lagring og handtering av importerte partier av rapslinje MS8, RF3 and MS8 x RF3 kan teoretisk representere et potensiale for utkryssing og spredning av transgener til dyrkede sorter og viltvoksende populasjoner i Norge. Forekomsten av disse genmodifiserte oljerapsplanter og sannsynligheten for introgresjon av genetisk materiale fra forvillet raps til nærstående, ville arter vurderes imidlertid til å være svært lav i et importscenario.

Samlet konklusjon

VKMs faggruppe for genmodifiserte organismer finner det lite trolig at den omsøkte bruken av oljerapslinjene MS8, RF3 og MS8 x RF3 vil medføre endret risiko for miljø i Norge sammenlignet med annen raps.

Abbreviations and explanations

ARMG	Antibiotic resistance marker gene
bar	bialaphos resistance, a gene encoding phosphinothricin-N-acetyltransferase gene, GA resistance gene
barnase	ribonuclease gene
barstar	gene coding for the inhibitor of Barnase, namely Barstar
BC	Backcross. Backcross breeding 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
canola	Term registered and adopted in Canada for oilseed rape with <2% erucic acid in the oil and <30 µmol/g glucosinolates in the air-dried, oil-free meal.
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)
CTP	Chloroplast transit peptide
DAP	Days after planting
DN	Norwegian Directorate for Nature Management (Direktoratet for naturforvalting)
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
E-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
Gluphosinate-ammonium	Broad-spectrum systemic herbicide

GM	Genetically modified
GMO	Genetically modified organism
GMP	Genetically modified plant
Ha	Hectare
HGT	Horizontal gene transfer
ILSI	International Life Sciences Institute
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
MS	Male sterility
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
pat	Phosphinothricin-Acetyl-Transferase (gene)
PAT	Phosphinothricin-Acetyl-Transferase (protein)
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
RF	Restoration of Fertility
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 *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. 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 *vir* genes of the Ti plasmid.
TA29	tapetum specific promoter
TI	Trait integration
U.S. EPA	United States Environmental Protection Agency.
Western blot	A procedure in which proteins separated by electrophoresis in polyacrylamide gels are transferred (blotted) onto nitrocellulose or nylon membranes and identified by specific antibodies.
WHO	World Health Organisation.

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Background

In preparation for the legal implementation of EU-regulation 1829/2003 in Norway, the Norwegian Scientific Committee for Food Safety has been requested by the Norwegian Directorate for Nature Management to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that the VKM already has conducted its final risk assessments on. However, the Directorate requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The genetically modified, glufosinate-tolerant oilseed rape lines MS8 (unique identifier ACS-BNØØ5-8), RF3 (unique identifier ACS-BNØØ3-6) and MS8 x RF3 (unique identifier ACS-BNØØ5-8 x ACS-BN003-6) (Notification C/BE/96/01) were approved for import and processing for animal feed and industrial purposes under Directive 2001/18/EC in 26 March 2007 (Commission Decision 2007/232/EC).

The VKM GMO Panel has previously issued a scientific opinion related to notification C/BE/96/01 for the placing on the market of the oilseed rape lines for import, processing and feed use (VKM 2008). The health and environmental risk assessment was commissioned by the Norwegian Directorate for Nature Management in connection with the national finalisation of the procedure of the notification C/BE/96/01 in 2008. Due to the publication of updated guidelines for environmental risk assessments of genetically modified plants and new scientific literature, the VKM GMO Panel has decided to deliver an updated environmental risk assessment of oilseed rape MS8, RF3 and MS8 x RF3.

The original application from Bayer CropScience (C/BE/96/01) was submitted to the Belgian Competent Authorities (CA) in 1996, with a request for placing on the market under the Directive 90/220/EEC, the male sterile MS8 line, the fertility restorer RF3 line and their hybrid MS8xRF3 for the purpose of cultivation, import and processing into animal feeding stuffs and industrial products. After evaluation of the notification by the competent Scientific Committee of the Belgian Biosafety Advisory Council, the Belgian CA forwarded the notification with a positive opinion to the European Commission in December 1996. In May 1998, the Scientific Committee on Plants concluded that there was no evidence to indicate that the placing on the market of oilseed rape MS8xRF3, with the purpose to be used as any other variety of oilseed rape, is likely to cause adverse effects on human health and the environment (SCP 1998).

With the entry into force of the Directive 2001/18/EC according to Article 35 of the Directive, Bayer CropScience submitted an update of the initial notification C/BE/96/01 to the Belgian CA in January 2001. The notifier provided additional demanded information to the CA in 2003, and on February 2, 2004 the Belgian Competent Authority forwarded its assessment report to the Commission. The assessment report concluded that consent for placing on the market should be granted for the following uses: import and processing of oilseed rape Ms8, RF3 and Ms8xRF3 and for its use as other any oilseed rape, excluding the cultivation in the EU of varieties derived from the oilseed rape events Ms8, RF3 and Ms8xRF3. The Belgian CA referred to potential loss of biodiversity due to the use of the associated herbicide (as demonstrated in the Farm Scale Evaluations) and that a number of the recommendations of the agricultural guidelines and measures proposed by the notifier in order to limit the vertical gene flow and its consequences are impracticable, hardly workable and hard to control in current agricultural practices. The EFSA GMO Panel published its scientific opinion on notification C/BE/96/01 14 September 2005 (EFSA 2005).

Processed oil from genetically modified oilseed rape derived from MS8, RF3 and MS8 x RF3 were notified as existing food according to Art. 5 of Regulation (EC) No 258/97 on novel foods and novel food ingredients in November 1999. Existing feed and feed products containing, consisting of or produced from MS8, RF3 and MS8 x RF3 were notified according to Article 20(1) b of Regulation

(EC) No 1829/2003 and were placed on the market in 2000 and registered in the Community Register in 2005 (CERA 2013).

An application for renewal of the authorisation for continued marketing of existing food, food ingredients and feed materials produced from MS8, RF3 and MS8 x RF3 was submitted within the framework of Regulation (EC) No 1829/2003 in June 2007 (EFSA/GMO/RX/MS8/RF3). In addition, an application covering food containing or consisting of, and food produced from or containing ingredients produced from oilseed rape MS8, RF3 and MS8 x RF3 (with the exception of processed oil) was delivered by Bayer CropScience in June 2010 (EFSA/GMO/BE/2010/81). The EFSA GMO Panel has assessed the two applications, and published its scientific opinions in 2009 and 2012, respectively (EFSA 2009a, 2012). A scientific opinion on the application EFSA/GMO/BE/2010/81 has also been submitted by the VKM GMO Panel (VKM 2012).

Through the Agreement of the European Economic Area (EEA), Norway is obliged to implement the EU regulations on GM food and feed (regulations 1829/2003, 1830/2003 et al). Until implementation of these regulations, Norway has a national legislation concerning processed GM food and feed products that are harmonised with the EU legislation. These national regulations entered into force 15 September 2005. For genetically modified feed and some categories of genetically modified food, no requirements of authorisation were required before this date. Such products that were lawfully placed on the Norwegian marked before the GM regulations entered into force, the so-called existing products, could be sold in a transitional period of three years when specific notifications were sent to the Norwegian Food Safety Authority. Within three years after 15. September 2005, applications for authorisation should be sent to the Authority before further marketing.

Four fish feed producing companies have once a year since 2008, applied for an exemption of the authorisation requirements of 19 existing products, including oilseed rape MS8, RF3 and MS8 x RF3. These 19 GM events are all authorised in the EU, and the Norwegian Food Safety Authority has granted exemption for a period of one year each time.

Terms of reference

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Directorate for Nature Management, by letter dated 13 June 2012 (ref. 2008/4367/ART-BI-BRH), requests the Norwegian Scientific Committee for Food Safety, to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act.

The request does not cover GMOs that the Committee already has conducted its final risk assessments on. However, the Directorate requests the Committee to consider whether updates or other changes to earlier submitted assessments are necessary.

The basis for evaluating the applicants' environmental risk assessments is embodied in the Act Relating to the Production and Use of Genetically Modified Organisms etc. (the Norwegian Gene Technology Act), Regulations relating to impact assessment pursuant to the 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 2010, 2011a), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

The risk assessments' primary geographical focus should be Norway, and the risk assessments should 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 changes in the use of pesticides.

Assessment

1 Introduction

The genetically modified oilseed rape lines MS8 and RF3 were developed to provide an effective pollination control system for production of F_1-hybrid seeds (MS8 x RF3). Oilseed rape is a crop capable of undergoing both self-pollination (approximately 70%) as well as cross-pollination (30%). Therefore a system to ensure only cross-pollination is required for producing hybrids from two distinct parents. As a result of hybrid vigor cross-pollinated plants produce higher yield and is more uniform as compared to self-pollinating rape.

The hybrid system is achieved using a pollination control system by insertion and expression of *barnase* and *barstar* genes derived from the common soil bacterium *Bacillus amyloliquefaciens* into two separate transgenic oilseed rape lines. The *barnase* gene in the male sterile line MS8 encode a ribonuclease peptide (RNase), expressed in the tapetum cells in the pollen sac in early stages of the anther development. The RNase effect RNA levels, disrupting normal cell function and arresting early anther development, and results in the lack of viable pollen and male sterility.

The fertility restoration line RF3 contains a *barstar* gene, coding for a ribonuclease inhibitor (Barstar peptide) expressed only in the tapetum cells of the pollen during anther development. The peptide specifically inhibits the Barnase RNase expressed by the MS8 line. The RNase and the ribonuclease inhibitor form a stable one-to-one complex, in which the RNase is inactivated. As a result, when pollen from the receptor line RF3 is crossed to the male sterile line MS8, the MS8 x RF3 progeny expresses the RNase inhibitor in the tapetum cells of the anthers allowing hybrid plants to develop normal anthers and restore fertility.

The *barnase* and *barstar* genes in MS8 and RF3 are each linked with the *bar* gene from *Streptomyces hygroscopus*. The *bar* gene is driven by a plant promoter that is active in all green tissues of the plant, and encodes the enzyme phosphinothricin acetyltransferase (PAT). The PAT enzyme detoxifies glufosinate-ammonium by acetylation of the L-isomer into N-acetyl-L-glufosinate ammonium (NAG) and therefore confers tolerance to the herbical active substance glufosinate ammonium. The *bar* gen were transferred to the oilseed rape plants as markers both for use during in vitro selection and as a breeding selection tool in seed production.

The genetically modified, glufosinate-tolerant oilseed rape lines MS8, RF3 and MS8 x RF3 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 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 oilseed rape MS8, RF3 and MS8 x RF3 is based on information provided in the applications EFSA/GMO/RX/MS8/RF3, EFSA/GMO/BE/2010/8, and C/BE/96/01, additional information obtained from the applicant and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

In line with its mandate, VKM emphasised 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.

2 Molecular characterisation

2.1. Evaluation of relevant scientific data

2.1.1 Transformation process and vector constructs

The oilseed rape line MS8xRF3 is a fertile hybrid derived through conventional breeding of the male sterile oilseed rape line MS8 and the oilseed rape line RF3, called the fertility restorer. MS8xRF3contains the *bar, barstar* and *barnase* genes, and is tolerant to glufosinate ammonium containing herbicides.

MS8 contains the *bar* and *barnase* gene, and RF3 contains the *bar* and *barstar* gene. The *barnase* and *barstar* genes have both been isolated from the bacterium *Bacillus amyloliquefaciens*. They code for two small single-chain proteins, designated as Barnase and Barstar, respectively. Under the control of a specific plant promoter that exclusively expresses these genes in the tapetal cell-layer during anther development, the *barnase* and *barstar* genes are the basis of a well-characterised hybridisation system in oilseed rape. The *bar* gene has been isolated from *Streptomyces hygroscopicus*, a microorganism that produces bialaphos. Bialaphos or its synthetically produced component glufosinate ammonium is a registered herbicide with phosphinothricin as the active ingredient. The *bar* gene product, PAT (phosphinothricin acetyl transferase), metabolises phosphinothricin to an inactive, acetylated derivative.

MS8 and RF3 oilseed rape were produced by *Agrobacterium tumefaciens* mediated transformation of hypocotyl explants of the *Brassica napus* cultivar Drakkar with the plasmids pTHW107 and pTHW118, respectively. The plasmids pTHW107 and pTHW118 are both part of a binary *A. tumefaciens* vector system. Both plasmids have identical backbone structures and have been specifically designed for the cloning of desirable expression cassettes for *A. tumefaciens* mediated transformation of oilseed rape.

A full description of the nature and source of the plasmids pTHW107 and pTHW118 is provided in the application EFSA-GMO-RX-MS8-RF3 (Part I, Section C.2., page 32). The inserted T-DNA within the MS8/RF3 does not add a bacterial origin of replication to the wild type *Brassica napus* genome. No other marker genes are present.

The genetic elements of the T-DNA components of pTHW107 and pTHW118 are described in Table 1 and Table 2.

Table 1. Genetic Elements of T-DNA Component of pTHW107 (MS8).

Nt Positions	Orientation	Origin
1-25		**RB:** right border repeat from the T-DNA of *Agrobacterium tumefaciens* (Zambryski 1988)
26-331	Counter clockwise	**3´g7:** sequence including the 3´ untranslated region of the TLDNA gene 7 of the *Agrobacterium tumefaciens* octopine Ti plasmid. (Dhaese et al. 1983)
332-883	Counter clockwise	***bar*:** the coding sequence of the phosphinothricin acetyltransferase gene of *Streptomyces hygroscopicus* as described by Thompson et al. (1987).
884-2658	Counter clockwise	**PssuAt:** sequence incuding the promoter region of the ribulose-1,5-biphosphate carboxylase small subunit gene of *Arabidopsis thaliana* as described by Krebbers et al. (1988)
2659-2919	Counter clockwise	**3´nos:** sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (Depicker et al.1982)
2920-3033	Counter clockwise	**3'barnase:** sequence including the 3' untranslated region of the *barnase* gene of *Bacillus amyloliquefaciens* (Hartley 1988)
3034-3369	Counter clockwise	***barnase*:** the coding sequence of the barnase gene of *Bacillus amyloliquifaciens* (Hartley 1988)
3370-4922	Counter clockwise	**Pta29:** sequence including the promoter of the anther-specific gene TA29 of *Nicotiana tabacum* (tobacco). (Seurinck et al.1990)
4923-4947		**LB:** left border repeat from the T-DNA of *Agrobacterium tumefaciens* (Zambryski, 1988)

Table 2. Genetic Elements of T-DNA Component of pTHW118 (RF3).

Nt Positions	Orientation	Origin
1-25		**RB:** right border repeat from the T-DNA of *Agrobacterium tumefaciens* (Zambryski 1988).
26-331	Counter clockwise	**3´g7:** sequence including the 3´ untranslated region of the TLDNA gene 7 of the *Agrobacterium tumefaciens* octopine Ti plasmid (Dhaese et al.1983).
332-883	Counter clockwise	***bar*:** the coding sequence of the phosphinothricin acetyltransferase gene of *Streptomyces hygroscopicus* as described by Thompson et al. (1987).
884-2658	Counter clockwise	**PssuAt:** sequence including the promoter region of the ribulose-1,5-biphosphate carboxylase small subunit gene of *Arabidopsis thaliana* as described by Krebbers et al. (1988).
2659-2981	Counter clockwise	**3´nos:** sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (Depicker et al. 1982).
2982-3254	Counter clockwise	***barstar*:** coding sequence of the *barstar* gene of *Bacillus amiloliquifaciens* as described by Heartley (1988).
3255-4808	Counter clockwise	**Pta29:** sequence including the promoter of the anther-specific gene TA29 of *Nicotiana tabacum* (tobacco). (Seurinck et al. 1990).
4809-4833		**LB:** left border repeat from the T-DNA of *Agrobacterium tumefaciens* (Zambryski, 1988).

2.1.2 Transgenic constructs in the genetically modified plant

2.1.2.1 Information on the sequences actually inserted or deleted

MS8 oilseed rape (male sterile line)
Southern blot analysis of MS8 oilseed rape genomic DNA has been carried out with a set of Southern probes spanning the entire length of the T-DNA region of plasmid pTHW107 in combination with different restriction digests. The applicant concludes that these Southern analyses demonstrate that MS8 oilseed rape contains a single copy of the pTHW107 T-DNA inserted at a single genomic locus, and that this was further confirmed by means of PCR analysis.

The absence of backbone sequences of plasmid pTHW107 in MS8 oilseed rape was evaluated by Southern blot analyses that were carried out with probes covering the complete backbone sequence of plasmid pTHW107. According to the applicant no hybridisation signals were observed for any of the Southern probes, thereby confirming the absence of plasmid THW107 backbone sequences in MS8 oilseed rape.

A complete description of the molecular characterization of MS8 oilseed rape is provided in application EFSA-GMO-RX-MS8-RF3. The inserted genetic elements in MS8 are described in Table 3. Amplification strategy is shown in Figure 1, and a physical map of the insert in Figure 2.

Table 3. Genetic elements of vector pTHW107 inserted into the plant genome of the male sterile line MS8.

Genetic elements of vector pTHW107 inserted into the plant genome of MS8	
PSsuAra	The promoter PSsuAra which has been isolated from *Arabidopsis thaliana.* The PSsuAra promoter regulates the expression of the *bar* gene. Its activity is most abundant in green tissues (leaves, stems and sepals).
Bar	The *bar* gene is isolated from the bacterium *Streptomyces hygroscopicus*, and encodes the phosphinothricin acetyl transferase (PAT) - enzyme
3'g7 (*3' TL7*)	Terminating signal from the TL-DNA gene 7 from *Agrobacterium tumefaciens.*
PTA29	The promoter TA29 of *Nicotiana tabacum,* regulates the expression of the *barnase* gene isolated from the bacterium *Bacillus amyloliquefaciens*. The TA29 promoter effectively limits the activity of the *barnase* gene in tissue (the tapetum cells of the pollen sac) as well as in time (only when flowering during anther development).
Barnase	Isolated from the bacterium *Bacillus amyloliquefaciens* encodes an extracellular ribonuclease (RNAse) capable of degrading and digesting RNA. Only expressed in the tapetum cells during anther development and results in lack of viable pollen and male sterility.
3'NOS	Part of the untranslated terminator sequence of the *nopalinesynthase-* gene from *Agrobacterium tumefaciens*.

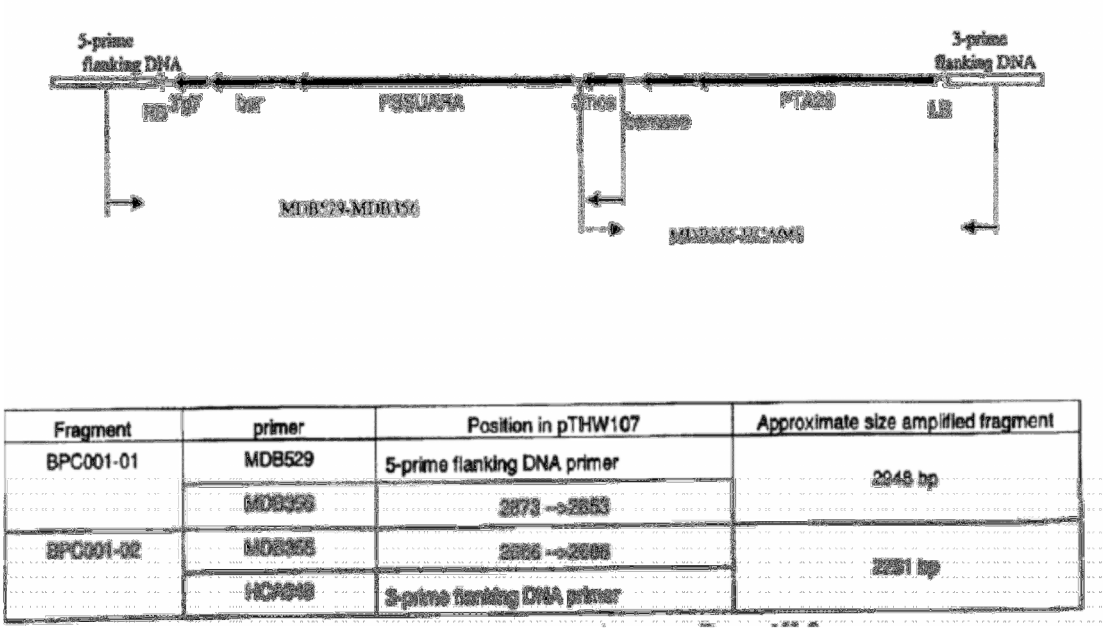

Figure 1. Amplification strategy – male sterile line MS8.

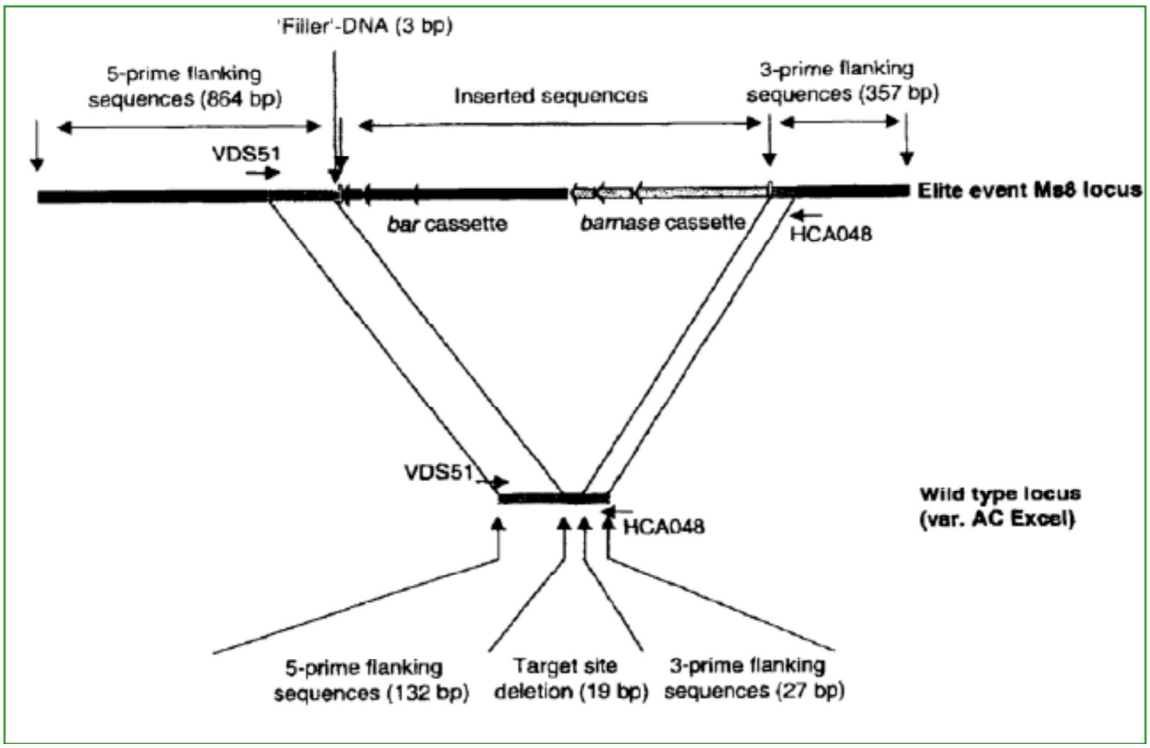

Figure 2. Physical map of the insert of event MS8 and schematic representation of the alignment of the MS8 transgene locus and the wild type locus.

RF3 oilseed rape (fertility restorer line)

Southern blot and PCR analyses of RF3 oilseed rape have demonstrated the presence of a single genomic locus that is composed of one partial copy of the pTHW118 T-DNA, flanked by another partial copy of the pTHW118 T-DNA in an inverted orientation. The inserted transgenic sequences in RF3 oilseed rape contain one partial copy of the T-DNA, consisting of a complete *bar* gene cassette and a *barstar* gene cassette containing only part of the Pta29 promoter, flanked by another partial T-DNA copy in an inverted orientation, which includes a complete *barstar* gene cassette and a part of the PssuAt promoter.

A detailed description of the RF3 molecular characterization has been provided as additional information to EFSA in January 2009 in the frame of application EFSA-GMO-RX-MS8-RF3.

The absence of backbone sequences of plasmid pTHW118 in RF3 oilseed rape has been evaluated by Southern blot and PCR analyses, together covering the complete backbone sequence of plasmid pTHW118. According to the applicant neither Southern hybridisation nor PCR amplification was detected for any of the Southern probes and PCR primer pairs, thereby confirming the absence of plasmid pTHW118 backbone sequences in RF3 oilseed rape. A detailed description of these studies is provided in application EFSA-GMO-RX-MS8-RF3.

The inserted genetic elements in RF3 are described in Table 4. Amplification strategy is shown in Figure 3 and a physical map of the insert in Figure 4.

Table 4. Genetic elements of vector pTHW118 inserted into the plant genome of the fertility restorer line RF3

Genetic elements of vector pTHW118 inserted into the plant genome of RF3	
PSsuAra::Bar:3' g7	The PSsuAra promoter regulates the expression of the *bar* gene (isolated from the bacterium *Streptomyces hygroscopicus*). Its activity is most abundant in green tissues (leaves, stems and sepals). Polyadenylation signals are provided by the 3'end of the T-DNA gene *7* of *Agrobacterium tumefaciens*.
PTA29:: Barstar: 3'NOS	The promoter TA29 of *Nicotiana tabacum* regulates the expression of the *barstar* gene of *Bacillus amyloliquefaciens*. Restores fertility to male sterile plants by inactivating the *barnase* gene. This sequence also contains the 3'end of the *nopalinesynthase* gene of *Agrobacterium tumefaciens*.

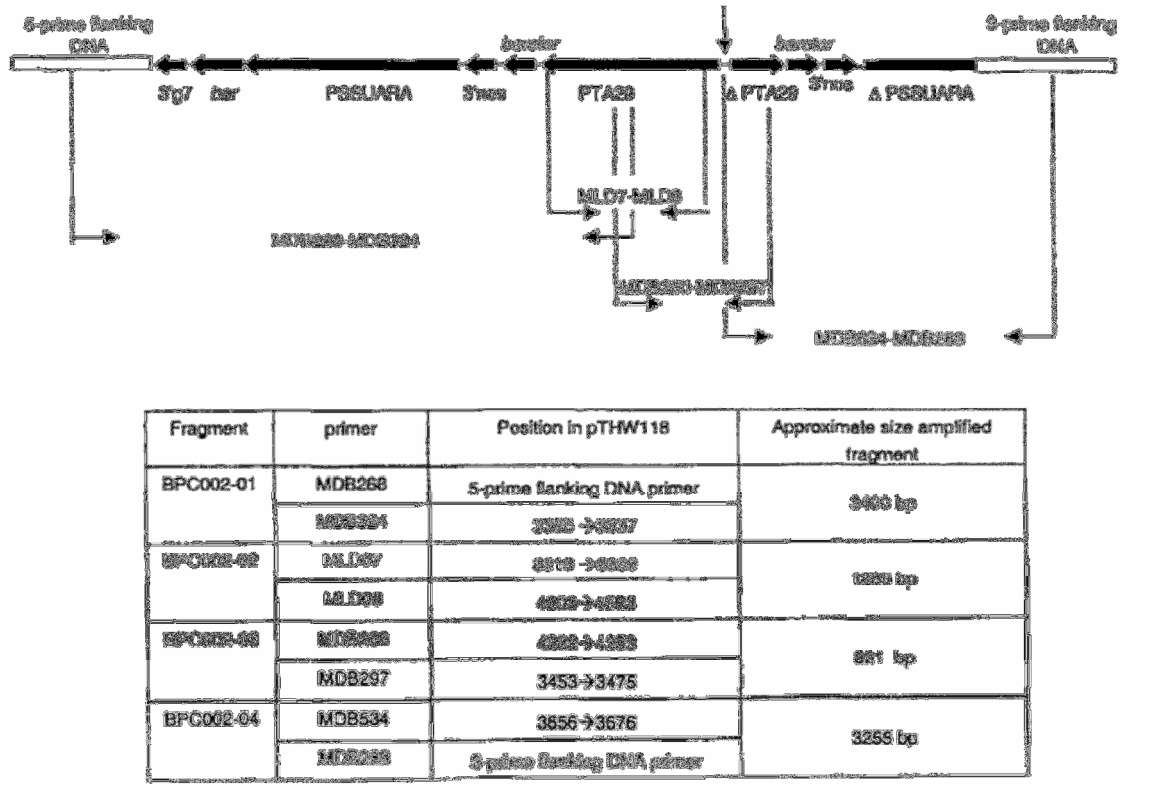

Figure 3. Amplification strategy, fertility restorer RF3.

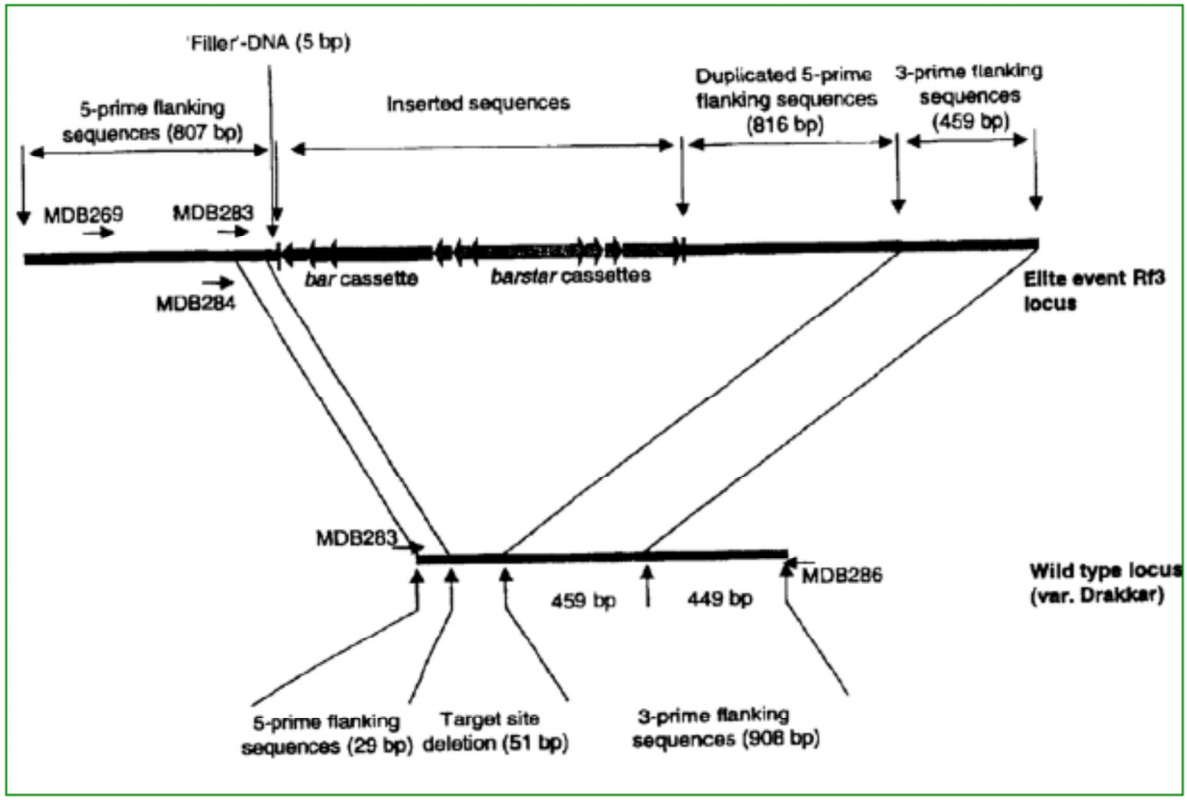

Figure 4. Physical map of the insert of event RF3 and schematic representation of the alignment of the RF3 transgene locus and the wild type locus.

2.1.3 Information on the expression of the inserts and open reading frames (ORFs)

Northern blot analyses have been performed on different tissues sampled at different developmental stages to demonstrate the expression of the introduced genes in MS8, RF3 and MS8xRF3 plants (Vandermarliere & De Beuckeleer 2004, unpublished). The results are summarised in Table 5.

The analyses showed *bar* gene expression in leaf and flower bud tissues of MS8 but no expression in seed or root tissues (detection limit < 1 pg gene transcript). The analysis in RF3 showed expression in leaf, root, flower buds and immature seed tissues but no expression in dry seeds (detection limit < 0.5 pg gene transcript). Likewise, the analyses showed that the *bar* gene was expressed in leaf, root, flower buds and immature seed tissues from the hybrid MS8xRF3, while no expression was observed in pollen or dry seeds (detection limit < 0,5 pg gene transcript). *Barnase* gene expression was not observed in any of the tested MS8 tissues. According to the applicant, the absence of detectable *barnase* gene expression in the flower buds from MS8 was most likely due to tapetal cell RNA hydrolysis by Barnase enzymatic activity.

Expression of the *barstar* gene was observed in flower buds sampled from RF3 plants, but was not detected in other tissues. According to the applicant this observation confirms temporal and spatial expression of the *barstar* gene.

Barnase and *barstar* expression analysis of tissues taken from the MS8xRF3 hybrid showed the expression of the *barnase* and the *barstar* genes in the flower buds. According to the applicant this was expected since it has been shown that the Barstar protein is able to complex efficiently with Barnase protein in anther tapetal cells and thus preventing the tapetal cell RNA hydrolysis.

According to the documentation the expression level of the *barstar* gene in the hybrid is approximately 10 times higher than the *barnase* expression levels in the MS8 or RF3 plants.

Table 5. Expression of the *bar-, barnase- and barstar*-genes in rape seed events MS8, RF3 and MS8xRF3.

Tissue	Line	Expression of *bar*-gene (pg/µg total RNA)	Expression of *barnase*-gene (pg/µg total RNA)	Expression of *barstar*-gene (pg/µg total RNA)
Young leaf	MS8 RF3 MS8xRF3	1.6-3.2 3.2-6.4 3.2	- ND -	ND - -
Mature leaf	MS8 RF3 MS8xRF3	3.2 3.2-6.4 3.2-6.4	- ND -	ND - -
Root	MS8 RF3 MS8xRF3	- 0.2 0.2	- ND -	ND - -
Flower bud	MS8 RF3 MS8xRF3	0.8 1.6 0.8-1.6	- ND 0.2-0.4	ND 3.2-6.4 3.2-6.4
Pollen	MS8 RF3 MS8xRF3	Not analysed - -	- ND -	Not analysed - -
Dry seed	MS8 RF3 MS8xRF3	- - -	- ND -	ND - -
Immature seed	MS8 RF3 MS8xRF3	Not analysed 0.2 0.2-0.4	- ND -	Not analysed - -

2.1.3.1 Protein expression

Western blot analyses of total protein extracts have been performed to check for the presence of Barnase, Barstar, Barnase/Barstar complex and/or PAT protein (Van der Klis 2004, unpublished). PAT expression was also confirmed by ELISA and a commercially available PAT protein test kit (strip test). The results are summarised in Table 6 and Table 7.

The data show that the PAT protein was detectable in all tissues but amounts were higher in green tissues and only at trace levels in others. Barstar was only detected in flower buds during pollen development in RF3 plants, while Barnase could not be detected in flower bud tissues of MS8 plants. According to the applicant this is most likely due to the highly specific expression, limited both temporally and spatially to the tapetal cell layer, and in addition the expression of the protein in this cell layer leads to the disruption of the tapetal cells. In flower bud tissues of MS8xRF3 plants, Barnase

and Barstar were detected under denaturing conditions. Under these conditions, the Barnase/Barstar protein complex dissociates into its two separate monomeric proteins Barnase and Barstar. Both proteins were recognised by the antibodies against the monomers of the complex.

Table 6. Results of Western blot

Tissue	Used anti-serum				
	Line	**anti-Barnase**	**anti-Barstar**	**anti-Barnase/ Barstar**	**anti-PAT**
Young leaf	MS8	-	Not analysed	Not analysed	+
	RF3	Not analysed	-	Not analysed	+
	MS8xRF3	-	-	-	+
	Wt	-	-	-	-
Root	MS8	-	Not analysed	Not analysed	+
	RF3	Not analysed	-	Not analysed	+
	MS8xRF3	-	-	-	+
	Wt	-	-	-	-
Mature leaf	MS8	-	Not analysed	-	+
	RF3	Not analysed	-	Not analysed	+
	MS8xRF3	-	-	-	+
	Wt	-	-	-	-
Flower bud	MS8	-	Not analysed	-	+
	RF3	Not analysed	+	Not analysed	+
	MS8xRF3	+*	+*	+*	+
	Wt	-	-	-	-
Pollen	RF3	Not analysed	-	Not analysed	+
	MS8xRF3	-	-	-	+
	Wt	-	-	-	-
Dry seed	MS8	-	Not analysed	-	+
	RF3	Not analysed	-	Not analysed	+
	MS8xRF3	-	-	-	+
	Wt	-	-	-	-

+: Detected
- : Below limit of detection
*: The Barnase/Barstar protein complex is dissociated under denaturing conditions and identified as separate monomers of Barnase and Barstar proteins

Table 7. Protein content and PAT ELISA results of different seed samples in comparison with the strip test.

WS	Pedigree	Event	Crushed seed gram	Protein mg/ml	PAT				STRIP
					µg/ml	µg/mg total protein	µg/g seed	% of total extractable protein	
WOSR	Parental line	MS8/ -	0.5074	21.80	0.036	0.002	0.07	0.0002	+
WOSR	Parental line	RF3/RF3	0.5172	20.58	0.080	0.004	0.15	0.0004	+
WOSR	F1	MS8/RF3	0.5053	19.96	0.076	0.004	0.15	0.0004	+
WOSR	F1	-/-	0.5077	20.32	ND[1]	ND	ND	ND	-
WOSR	F2	MS8/RF3	0.5109	13.99	0.060	0.004	0.12	0.004	+
WOSR	F2	-/-	0.5101	14.92	ND	ND	ND	ND	-
SOSR	Parental line	MS8/-	0.5023	26.05	0.049	0.002	0.10	0.0002	+
SOSR	Parental line	RF3/RF3	0.4150	15.73	0.129	0.008	0.31	0.0008	+
SOSR	Parental line	-/-	0.5005	20.57	ND	ND	ND	ND	-
SOSR	F1	MS8/RF3	0.5044	14.78	0.112	0.008	0.22	0.0008	+
SOSR	F2	MS8/RF3	0.5112	17.26	0.057	0.003	0.11	0.0003	+

[1] ND -Not detectable

2.1.3.2 Open reading frames (ORFs)

According to the applicant, bioinformatic examination of the gene insertion site, the flanking regions and the plant DNA junctions has shown that the integration sequences of oilseed rape event MS8 and RF3 can be assumed as not being transcriptionally active and all predicted cryptic ORFs can be considered as not biologically meaningful. According to the applicant it has also been demonstrated that the putative nucleotide ORF sequences do not code for proteins which have potential toxic or allergenic properties.

The Right Border and Left Border junction sequences of events MS8 and RF3 have been determined and according to the applicant, an *in silico* analysis did not produce evidence that novel transcripts might arise at either junction of the oilseed rape MS8 and RF3 inserts. To demonstrate the presence / absence of cryptic gene expression from incoming and outgoing messages at the junctions of events MS8 and RF3, northern blot analysis have been performed on different tissues at different developmental stages of MS8xRF3 hybrid plants. According to the applicant, the analysis showed no positive signal in any of the tested tissues with the different cryptic RNA probes (detection limits varying between 0.25 pg and 1pg of the relevant transcripts).

The applicant concludes that the study characterises the presence of *bar* mRNA in various tissues of MS8, RF3 and MS8xRF3, that it confirms that the spatial and temporal expression of *barnase* and *barstar* genes is restricted to the flower buds, and that the genetic modification in MS8, RF3 and MS8 x RF3 does not lead to the detection of cryptic RNA transcript synthesis (Vandermarliere & De Beuckeleer 2004, unpublished).

Updated information

MS8
Bioinformatic analyses of the 5´ and 3´ flanking sequences of the MS8 insert were updated in 2008 (Additional information provided to EFSA in January 2009 in the frame of application EFSA-GMO-RX-MS8-RF3). Analysis of the 5' and 3' flanking regions using BLASTn and BLASTx did not identify any endogenous genes that could be interrupted or whose expression would be influenced due to the insertion of the T-DNA in MS8 oilseed rape.

To assess the presence of potential newly created coding sequences spanning the MS8 insert – genomic DNA junction regions, bioinformatic analyses using open reading frame (ORF) and gene search tools have been performed. This analysis was carried out to predict putative ORFs with a minimum size of three amino acids between start and stop codons and between two stop codons in all six reading frames. No indication was found of transcription of any new ORFs in MS8 oilseed rape. Furthermore, identified putative ORF translated amino acid sequences were subsequently compared with sequences of known toxins or known allergens contained in up-to-date versions of the Uniprot_Swissprot, Uniprot_TrEMBL, PDB, DAD, GenPept, and an in-house allergen databases, by using BLASTP or FindPatterns algorithms. According to the applicant the putative ORF amino acid sequences do not present any biologically significant sequence similarities with known toxins and known allergens.

RF3
Bioinformatic analyses of the 5´ and 3´ flanking sequences of the RF3 insert were updated in 2008 (Additional information provided to EFSA in January 2009 in the frame of application EFSA-GMO-RX-MS8-RF3). Analysis of the 5' and 3' flanking regions using BLASTn and BLASTx did not identify any endogenous genes that could be interrupted or whose expression would be influenced due to the insertion of the T-DNA in RF3 oilseed rape.

To assess the presence of potential newly created coding sequences in the junction regions spanning all four newly created junctions of the RF3 insert, bioinformatic analyses using open reading frame (ORF) and gene search tools were performed. This analysis was carried out to predict putative ORFs with a minimum size of three amino acids between start and stop codons and between two stop codons in all six reading frames. No indication was found of transcription of any new ORFs in RF3 oilseed rape. Furthermore, identified putative ORF translated amino acid sequences were subsequently compared with sequences of known toxins or known allergens contained in up-to-date versions of the Uniprot_Swissprot, Uniprot_TrEMBL, PDB, DAD, GenPept, and an in-house allergen databases, by using BLASTP or FindPatterns algorithms. According to the applicant the putative ORF amino acid

sequences do not present any biologically significant sequence similarities with known toxins and known allergens.

2.1.4 Inheritance and stability of inserted DNA

Southern blot analyses have demonstrated that the integrity of the inserts in the single events in MS8 and RF3 are preserved in the hybrid MS8xRF3. Observations in several field trials and extensive cultivation in North America and Canada show no alteration in the plant's phenotype, and analyses have shown that the hybrid system MS8/RF3 is stably expressed over multiple generations, independent of genotype, generation or environment. The traits are expressed in a predictable and stable manner, at the appropriate development stage and throughout the growth cycle.

MS8/RF3 derived lines and varieties have been grown in Canada since 2000, and have displayed consistent tolerance to herbicides with glufosinate ammonium.

2.2 Conclusion

The oilseed rape hybrid MS8xRF3 is produced by conventional crossing. The parental lines MS8 (male sterile) and RF3 (fertility restorer) are well described in the documentation provided by the applicant, and a number of publications support their data. It seems likely that MS8 contains a complete copy of the desired T-DNA construct including the *bar* and *barnase* genes. Likewise, the event RF3 is likely to contain complete copies of the *bar* and *barstar* genes in addition to a second incomplete non-functional copy of the *bar*-gene. The inserts in the single events are preserved in the hybrid MS8xRF3, and the desired traits are stably inherited over generations.

Oilseed rape MS8/RF3 and the physical, chemical and functional characteristics of the proteins have previously been evaluated by the VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2008). The VKM GMO Panel finds the characterisation of the physical, chemical and functional properties of the recombinant inserts in the oilseed rape transformation events MS8, RF3 and MS8xRF3 to be satisfactory. The GMO Panel has not identified any novel risks associated with the modified plants based on the molecular characterisation of the inserts. This view is shared by the EFSA GMO Panel which has previously given positive scientific opinions on MS8, RF3 and MS8xRF3 oilseed rape (EFSA 2005, 2009a, 2012).

3 Production, import and use of oilseed rape

Oilseed production
The worldwide production of oilseed rape in 2011 was about 33.5 million hectares (ha) (FAOSTAT 2013). The production is greatest in Canada (7.5 mill ha), China (7.3 mill ha) and India (6.5 mill ha). In Europe, oilseed rape was harvested from 8.5 million ha in 2011 (EU-27 6.7 million ha), with the greatest production in France, Germany, UK and Poland. Total EU production of rapeseed in 2011 was approximately 28.5 million tonnes (FAOSTAT 2013).

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; Gain Report 2012). It is estimated that 3.4 million tonnes of rapeseed will be imported during the 2012-13 season, an increase of nearly 1 million tonnes from 2009/2010 (EU-COM 2013). The majority of rapeseed imports to the EU come from Australia, Ukraine and Canada (Gain Report 2011).

In Norway, the acreage used for cultivation of oilseed rape has varied significantly during the past 15 years (Statistics Norway 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 2, Appendix 1).

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.

The Norwegian imports of rapeseed oil in 2007 amounted to 1,136,431 tonnes (SLF 2008). With the exception of the Norwegian company “Norsk Matraps BA”, there is no industrial processing of oilseed in Norway (G. Sandvik, SLF, pers. comm.). “Norsk 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.

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 main by-products from oil-processing, is used as feed for all classes of livestock. 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 2013). According to statistics from the Norwegian Agricultural Authority, 100 100 tonnes of processed rapeseed (pellets/meal) were imported in 2012 as a raw protein product for use in the Norwegian feed concentrate production (SLF 2013). Similarly, 6900 000 tonnes of oilseeds were imported for production of concentrate feeds. 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 2012, 8800 tonnes of Norwegian-produced oilseeds were used for the

production of feed (SLF 2013). According to Hoel et al. (2013), the total production of oilseeds in Norway in 2012 were anticipated to 8 000 tonnes.

Forage rape varieties 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 transhipment 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 probability 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 therefor 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 feed production (rapeseed pellets, meal and cakes not included).

4 Comparative assessment

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

The transgenic oilseed rape lines MS8, RF3 and derived hybrids MS8 x RF3 have been extensively field tested in Canada (1994-1996, 2008) and Europe (1996,1997, 2001,2002) (Technical dossier applications C/BE/96/01, EFSA/GMO/RX/MS8/RF3, EFSA/GMO/BE/2010/81). The agronomic and phenotypic data on oilseed rape MS8xRF3 have been previously evaluated by the VKM GMO Panel (VKM 2008, 2012 unpublished).

4.1.1 Experimental design & statistical analysis

In the notification for placing on the market of MS8 x RF3 under Part C of Directive 2001/18 (C/BE/96/01) and the renewal application from 2008 (EFSA/GMO/RX/MS8/RF8) the applicant present data from compositional assessment in Belgium in the growth seasons 2001 and 2002.

In these field trials, MS8, RF3 and MS8 x RF3 were compared with a conventional counterpart having a comparable genetic background, i.e. the open pollinated winter oilseed rape line named PP0005B. The commercial spring oilseed rape variety “Drakkar”, was used as the recipient for the DNA insertion to establish transformation event MS8 and RF3, and were backcrossed into PP0005B using conventional backcrossing techniques. The MS8 event was backcrossed to PP005B until BC6 (7 crosses), while the RF3 event was backcrossed to PP005B until BC4 (5 crosses) and then subjected to 3 selfings to produce a homozygous RF3.RF3 PP005B parental line. Even with this level of backcrossing genotype conversion is not 100%, which means that the GM lines and the comparator are not fully isogenic.

Since the comparator is an open pollinated variety, the measured value is an average value that may be different from the values that would be measured in each individual. During the backcrossing procedure, a number of PP005B individuals are selected as the recurrent parents for the subsequent crosses. During the backcrossing it is not possible to select individuals randomly from the population in sufficient quantities to fully represent the population. This selection, over generations, can skew a set of recurrent parents (genetic drift) away from the original population phenotype. Therefore, in practice, a partially-inbred line is compared with a population. This bias has not great consequences with parameters that are relatively stable throughout the population but can be a problem with parameters that show great internal variation.

No conventional commercial reference varieties were included in the comparative assessments.

The trials were performed at 12 separate locations in Belgium, and distributed across a wide geographical area to provide a variety of agronomic practices, soils and climatic factors. At all sites, oilseed rape MS8 x RF3 and the conventional counterpart were planted following a complete randomized block design with four replicates per site. The plot size was 10m² and seeds were planted in 6 rows per plot. Glufosinate-ammonium (GA) was applied to predetermined plots at each site. (Treatment A stands for the non-transgenic control conventionally treated, treatment B stands for transgenic LL OSR MS8xRF3 conventionally treated and treatment C stands for transgenic LL OSR MS8xRF3 treated with glufosinate ammonium (Liberty)). The first GA application was made at the 2-4 leaf stage and the second application prior to winter or early spring with a dosage of 4.0 l/ha and only on the treated blocks of the transgenic plots. All plots were harvested at maturity.

In the documentation submitted by the applicant, means, analysis of variance (ANOVA), the coefficient of variation (C.V.) and the Least Significant Difference (LSD) are tabulated within each year for each analysed characteristics. No combined analyses of variance over years are presented.

4.2 Agronomic traits and GM phenotype

During field trials conducted over two growth seasons and different locations, MS8xRF3 and its non-transgenic counterpart were monitored from germination until harvest for a number of agronomic and phenotypic parameters. According to the applicant, data on plant morphology (plant height, maturity, lodging resistance), field performance (establishment, vigour, height, rate of growth), productivity (seed yield), disease susceptibility, preproduction, fecundity and persistence were collected (Table 8-13).

Table 8. Summary of parameters evaluated in the comparison of MS8/RF3 and the recipient variety cv. Drakkar in the Belgian field trials (2001/2002).

Characteristics	Parameters
Plant morphology	Plant height, maturity, lodging resistance, seed yield
Seed characteristics	Oil content, protein content, alkenyl content
Field performance	Establishment, vigour, height, rate of growth (days to 50 % bloom)
Productivity	Seed yield
Disease susceptibility	Severity rating for naturally occurring pathogens
Reproduction	Flower morphology, days to 50% flowering, days to finish flowering, days to maturity
Fecundity	Seed yield
Persistence	Competing ability, invasive potential
Nutritional composition of seed	Proximates (moisture, total fat, total protein, ash, total carbohydrates, crude fibre, ADF, NDF), amino acids, minerals, fatty acids, vitamin E
Antinutritional components	Glucosinolates, erucic acid, phytic acid

Mean values for the different agronomic data from the growth seasons 2001-2002 are summarised in tables 10-13. The coefficient of variation (CV) and LSD-values are also tabulated. There were no significant differences between the entries in treated block and the non-transgenic counterpart for all agronomic parameters except for the vigour after herbicide treatment. Following GA treatment, the MS8xRF3 hybrid demonstrated a temporary reduction in vigour relative to the untreated MS8xRF3 and the non-transgenic counterpart. This vigour reduction quickly disappeared, and was no longer apparent at the onset of flowering.

Table 9. Summary of phenotypic and agronomic parameters evaluated in MS8/RF3 and cv. Drakkar in the Belgian field trials (2001/2002).

Character	Abbreviation	Stage	Scale	Scale details		
				1	5	9
Date of seeding	DOS	1	Date			
Establishment	EST	12	(1-9)	Very thin	Average	Very thick
Vigour before GA treatment	VIG_bb	12	(1-9)	Poor	Average	Vigorous
Vigour after GA treatment	VIG ab	14	(1-9)	Poor	Average	Vigorous
Flowering – start (90% in flower)	FLST	61	(1-9)	Late	Average	Early
Flowering –end (10% remains in flower)	FLEN	69	(1-9)	Late	Average	early
Plant height	HEI	75	(1-9)	Very short		Very tall
Lodging resistance at maturity	LOM	85	(1-9)	0 degrees (flat)	45 degrees	90 degrees (upright)
Maturity	MAT	85	(1-9)	late	average	early
Date of harvest	DOH	99	Date			
Plot yield	YLDP	99	gram			
Yield/ha (9% moisture)	YLD(9)	99	kg/ha			

Table 10. Mean agronomic data from the 2001 growth season.

Parameter	EST	VIG-bb	VIG-ab	FLST	FLND	HEI	MAT	LOM	YLD (9)
Entries Scale	**1-9**	**1-9**	**1-9**	**1-9**	**1-9**	**cm**	**1-9**	**1-9**	**Kg/ha**
1 Treated Block	6.13	4.96	5.46	5.25	5.00	4.88	5.00	8.42	3661.71
2 Treated Block	5.79	4.96	5.96	5.29	5.00	4.83	5.00	8.46	3798.71
3 Treated Block	6.00	5.04	5.83	5.29	5.00	4.79	5.00	8.42	3735.20
1 Untreated block	6.13	4.96	5.75	5.25	4.83	4.96	4.83	7.92	3752.49
2 Untreated block	5.79	4.96	5.96	5.29	4.83	4.92	4.83	8.25	3820.59
3 Untreated block	6.00	5.04	5.83	5.29	4.83	4.79	4.83	8.08	3781.25
Mean	5.79	4.99	5.80	5.28	4.92	4.86	4.92	8.26	3758.32
LSD (5%)	0.35	0.18	0.27	0.06	-----	0.11	-------	0.24	199.36
V.C. (%)	10.39	6.34	8.14	2.00	-----	3.96	-------	5.02	9.28

Table 11. Percent mean of the checks/conventional comparator (2001 growth season).

Parameter	EST	VIG-bb	VIG-ab	FLST	FLND	HEI	MAT	LOM	YLD (9)
Entries % of comp.	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
1 Treated Block	103.9	99.2	96.2	99.2	101.7	10.9	101.7	101.4	96.8
2 Treated Block	98.2	99.2	101.1	100.0	101.7	100.0	101.7	101.9	100.4
3 Treated Block	101.8	100.8	98.9	100.0	101.7	99.1	101.7	101.4	98.7
1 Untreated block	103.9	99.2	97.5	99.2	98.3	102.6	98.3	95.4	99.2
2 Untreated block	98.2	99.2	101.1	100.0	98.3	101.7	98.3	99.4	100.9
3 Untreated block	101.8	100.8	98.9	100.0	98.3	99.1	98.3	97.4	99.9
Mean	101.3	99.7	98.4	99.7	100.0	100.6	100.0	99.5	99.3
LSD (5%)	6.0	3.6	4.6	1.1	--------	2.3	-------	2.9	5.3
V.C. (%)	10.4	6.3	8.1	2.0	--------	3.9	-------	5.0	9.3

Table 12. Mean agronomic data from the 2002 growth season.

Parameter	EST	VIG-bb	VIG-ab	FLST	FLND	HEI	MAT	LOM	YLD (9)
Entries Scale	**1-9**	**1-9**	**1-9**	**1-9**	**1-9**	**1-9**	**1-9**	**1-9**	**Kg/ha**
1 Treated Block	6.58	4.96	4.25	5.00	5.00	5.00	5.00	8.00	4299
2 Treated Block	6.71	4.96	4.96	5.00	5.00	5.00	4.96	8.00	4268
1 Untreated block	6.54	5.00	4.96	5.25	5.00	4.96	4.96	8.00	4270
2 Untreated block	6.58	5.00	4.96	5.29	5.00	4.79	4.96	8.04	4296
Mean	6.60	4.98	4.97	5.28	5.00	4.86	4.98	8.01	4278
LSD (5%)	0.16	------	0.04	0.06	------	0.11	-----	0.04	105
V.C. (%)	5.78	-------	2.05	2.00	------	3.96	-----	1.27	5.97

Table 13. Percent mean of the checks/conventional comparator (2002 growth season).

Parameter	EST	VIG-bb	VIG-ab	FLST	FLND	HEI	MAT	LOM	YLD (9)
Entries % of comp.	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
1 Treated Block	98.1	100	86.7	100	100	100	100.8	100	100.7
2 Treated Block	100	100	100	100	100	100	100	100	100
1 Untreated block	99.4	100	100	99.2	100	103.5	100	99.5	99.9
2 Untreated block	100	100	100	100	100	100	100	100	100
Mean	99.4	100	96.4	99.8	100	101.0	100.2	99.9	100.2
LSD (5%)	2.42	-----	1.64	0.80	-----	2.26	-----	0.50	2.46
V.C. (%)	5.78	-----	2.05	2.00	-----	3.96	-----	1.27	5.97

The stability of oilseed rape MS8, RF3 and MS8 x RF3 and the parental line cv. Drakkar has also been evaluated in field trials in Europe (Sweden, Belgium, France, UK) and Canada in 1996 and 1997 (12 field sites) (Technical Dossier: Weston 1998). The field trials were designed as a complete randomized block design with 3 or 4 repetitions, and the transgenic entries were sprayed with glufosinate ammonium at the four leaf stage. The following parameters were investigated: emergence and establishment, segregation, GA tolerance, vigour, flowering date, male sterility and restored fertility, stability of sterility throughout season and under different climatic conditions, female fertility and seed set, plant morphology, maturity, yield and seed quality parameters.

According to Weston (1998) the emerge of MS8/RF3 and the non-transgenic entries were comparable, and no important differences in vigour were observed between the different entries. No significant differences in plant height and yield were observed between MS8/RF3 and the control. In the 1996 growth season, the MS8 x RF3 restored hybrid was essentially equivalent to cv. Drakkar for earliness in flowering. In 1997, the MS8 x RF3 restored hybrid line was slightly earlier to flower than Drakkar. The MS8 line flowered as early as the comparator, while RF3 flowered 2 days later than cv. Drakkar. In Sweden, all three test lines (MS8, RF3 and MS8 x RF3 hybrids) flowered earlier than the control in 1997. The MS8 and RF3 lines were, however, later to mature (2 to 5 days, depending on location) while the MS8 x RF3 restored hybrid line had equivalent if not earlier maturity than cv. Drakkar in all locations in 1996.

The applicant also notes that throughout the field testing history and the commercial cultivation of oilseed rape MS8/RF3 in Canada since 2000, there have been observed no differences that could be attributed to pleiotropic effects of the *bar* gene insertion. Neither did MS8, RF3 and MS8xRF3 differ from the recipient in nutritional, agronomic or reproductive characters, except for vigour after herbicide treatment which disappeared quickly and was no longer apparent at the onset of flowering.

4.3 Conclusion

Based on results from comparative analyses of data from field trials located at representative sites and environments in Europe and Canada, and it is concluded that oilseed rape MS8, RF3 and MS8 x RF3 is agronomically and phenotypically equivalent to the conventional counterpart, except for the newly expressed barnase, barstar and PAT proteins.

The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glufosinate herbicide do not alter the phenotypic and agronomic characteristics of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape.

5 Environmental risk assessment

The notification C/BE/96/01 under Part C of Directive 2001/18 is for the authorisation of genetically modified oilseed rape MS8, RF3 and MS8 x RF3 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 EFSAS 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 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 MS8/RF3.

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, AACC, originating from a interspecific hybridization between the two diploid species *B. oleracea* L. (2n =18, CC) and *B. rapa* L. (2n = 20, AA) (OECD 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 MS8 line is male sterile and will therefore not pollinate any other plants. Although these plants can act as pollen recipients, their progeny will also be male sterile and will not produce pollen. The RF3 and MS8 x RF3 hybrid plants displayed normal reproductive characteristics.

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 melifera*) are an important insect pollinator of oilseed rape in Scandinavia, followed by bumblebees (*Bombus* sp.), 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 2008; 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 & Lutman 1998; Gulden et al. 2003). In a European study with winter oilseed rape, seeds buried immediately ofter 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 germinal to an ungerminal state and no seedling recruitment was observed. However, dormant oilseed rape seed has been found in tillage systems with low or no soil disturbance, indicating that rape seed can fall dormant at the soil surface even under light conditions (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; Crawley et al. 2001).

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, some studies 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 roadside feral populations containing 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 km^2). In the Danish study, 12 flowering plants/km^2 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/km^2) (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 (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 the 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 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 conventionally bred 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 to suggest that tolerance to glufosinate-ammonium or glyphosate 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. Progeny from hybrids of oilseed rape and wild relatives that bear the herbicide tolerant trait do not show any enhanced fitness, persistence and invasiveness, and behave as conventional counterparts, unless the herbicides for which tolerance is obtained are applied (Londo et al. 2010)

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 lines MS8, RF3 and MS8 x RF3 in Canada and Europe have shown equivalence between the transgenic lines and the corresponding, unmodified control with respect to agronomic and phenotypic characteristics. With the exception of tolerance to glufosinate ammonium, 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. Studies of seed quality parameters indicate no unintended effects of the introduced characteristics on the phenotypic characteristics of MS8, RF3 and MS8 x RF3.

Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 for reasons of reproductive toxicity.

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The genes coding for male sterility and fertility restoration do not confer any ecological advantage to potential hybrid offspring of MS8 or RF3 plants.

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 MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3.

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 2004, 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 MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3 in bacterial recipients.

In conclusion, the VKM GMO Panel consider it is unlikely that genes from MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3 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 volunteers 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; 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) (Lid & Lid 2005).

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 12 (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 12. Interspecific and intergeneric sexual crossing attempts, degree of success and potential for gene introgression[1] (Source: OECD 2011).

Interspecific cross	Sexual cross	Field cross	Seeds/ cross	BC (male)	BC (female)	Potential		References
						Natural cross	Intro-gression	
Brassica napus								
B.napus x B. juncea *B. juncea x B.napus*	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
B. napus x B. nigra *B .nigra x B.napus*	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
B. napus x B. oleracea *B. oleracea x B. napus*	Y							Gupta 1997
B. napus x B. rapa *B. rapa x B. napus*	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;
B.napus x B. adpressa *B. adpressa x B. napus*	Y Y	Y Y	2	Y	Y	H	L	Lefol et al. 1991, 1995, 1996b; Eber et al. 1994; Chevré et al. 1996
B. napus x B. tournefortii *B. tournefortii x B. napus*	Y F	NR	0.69			L VL	L VL	Nagpal et al. 1996; Gupta 1997; Salisbury 2002
B. napus x Diplotaxis muralis *D. muralis x B. napus*	Y NR	NR NR	0.28			L	VL	Bijral & Sharma 1996a
B. napus x D. erucoides *D. erucoides x B. napus*	NR Y	NR NR		Y		VK	VL	Ringdal et al. 1987
	Y	Y	$10^{-4, -8}$	Y	Y	H	L	Darmency et al. 1998; Eber et

B. napus x Raphanus raphanistrum *R. raphanistrum x B. napus*	Y	F						al. 1994; Lefol et al. 1997; Rieger et al. 1999; Chevré et al. 1997a, 1998
B. napus x R. sativus *R. sativus x B. napus*	Y NR	NR	0					Gupta 1997; Ammitzbøll & Jørgensen 2006
R. napus x Eruca sativa *E. sativa x B. napus*	Y NR	NR NR				L	VL	Birjal & Sharma 1996b
B. napus x Erucastrum gallicum *E. gallicum x B. napus*	Y F	F NR	0.1 0	Y	Y	VL VL	VL VL	Lefol et al. 1997; Warwick et al. 2003
B. napus x Sinapis alba *S. alba x B. napus*	Y F	NR NR	Y			VL EL	VL EL	Chevré et al. 1994; Brown et al. 1997
B. napus x S. arvensis *S. arvensis x B. napus*	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 el. 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; 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 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 (F_1-hybrid), while almost half of the plants had specific genetic markers from both *B. napus* and *B. rapa*. An 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 F_1-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 thos 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 F_1-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.

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 F_1 hybrids with *B. napus* (Lefol et al. 1996). The introgression of *B. napus* genes into *B. adpressa* is, however, not likely to be a 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 (Gueritaine 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 hybrids have 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 2008). 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 2008). *B. rapa* is also an unlikely 'intermediate host', as the F_1-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 MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3 with the biotic environment will be very limited. Some accidental spillage of seed from MS8, RF3 and MS8 x RF3 may however occur along transportation routes, processing plants and storing facilities during import, handling, storage and processing. PAT 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 MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3 is negligible.

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

Cultivation of oilseed rape MS8, RF3 and MS8 x RF3 in the EU is not included in the scope of the application C/BE/96/01. An assessment of the impacts of altered cultivation, management and harvesting techniques of MS8, RF3 and MS8 x RF3 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 oiseed rape MS8, RF3 and MS8 x RF3, and no deliberate release of viable plant material is expected in the EU/EEA and interactions of MS8, RF3 and MS8 x RF3 with the biotic environment will be very limited. The limited routes of exposure of soil micro-organisms to MS8, RF3 and MS8 x RF3 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 faces of livestock fed MS8, RF3 and MS8 x RF3. The likelihood of exposure of soil micro-organism to active PAT protein via manure and faeces of livestock fed with processed or unprocessed seed of MS8, RF3 and MS8 x RF3 is

negligible. PAT 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 MS8, RF3 and MS8 x RF3 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 notification C/BE/96/01 is the authorisation of MS8, RF3 and MS8 x RF3 for import, processing and the use of feed produced from MS8, RF3 and MS8 x RF3 in the EU under Directive 2001/18/EC.

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 MS8, RF3 and MS8 x RF3 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. The applicant has therefore considered that there is no need for case-specific monitoring.

Due to the limited exposure, and this only at import facilities or processing plants, it is unlikely that a possible spill of oilseed rape MS8, RF3 and MS8 x RF3 will have any influence on human or animal health or the environment.

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 are: (i) 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 are correct; and (ii) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment. Monitoring is related to risk management, and the final adoption of the monitoring plan falls outside the mandate of the Norwegian Scientific Committee.

However, the VKM GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant. In the context of the intended uses of MS8, RF3 and MS8 x RF3, 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 MS8, RF3 and MS8 x RF3 destined for processing into animal feed or industrial uses. The scope of the monitoring plan provided by the applicant is in line with the intended uses of oilseed rape MS8, RF3 and MS8 x RF3. As the scope of the notification does not include cultivation, the environmental risk assessment was concerned with the accidental release into the environment of viable seeds of oilseed rape MS8, RF3 and MS8 x RF3 unintentionally present in food, and with the horizontal gene transfer to bacteria occurring in the environment or human digestive tract. The environmental risk assessment identified no potential adverse effects to the environment, and no case-specific monitoring is necessary.

The general surveillance plan proposed by the applicant includes: (i) the description of an approach involving operators (federations involved in oilseed rape import and processing) reporting to the applicant via a centralised system any observed adverse effect(s) of GMOs on human health and the environment; (ii) a coordinating system for the collection of the information recorded by the various operators; and (iii) the use of networks of existing surveillance systems. The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent period.

The VKM GMO Panel considers that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of oilseed rape MS8, RF3 and MS8 x RF3, as the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects.

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 viability of rape seeds in commodities of whole oilseed rape imported for production of concentrate feeds.
- The presence, number and viability of rape seeds in the meal and cake from the crushing process and in the waste from cleaning operations.

Conclusion

Molecular characterisation

The oilseed rape hybrid MS8 x RF3 is produced by conventional crossing. The parental lines MS8 and RF3 are well described in the documentation provided by the applicant, and a number of publications support their data. It seems likely that MS8 contains a complete copy of the desired T-DNA construct including the *bar* and *barnase* genes. Likewise, the event RF3 is likely to contain complete copies of the *bar* and *barstar* genes in addition to a second incomplete non-functional copy of the *bar*-gene. The inserts in the single events are preserved in the hybrid MS8xRF3, and the desired traits are stably inherited over generations.

Oilseed rape MS8/RF3 and the physical, chemical and functional characteristics of the newly expressed proteins have previously been evaluated by the VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2008, 2012). The GMO Panel finds the characterisation of the physical, chemical and functional properties of the recombinant inserts in the oilseed rape transformation events MS8, RF3 and MS8xRF3 to be satisfactory. The GMO Panel has not identified any novel risks associated with the modified plants based on the molecular characterisation of the inserts.

Comparative assessment

Based on results from comparative analyses of data from field trials located at representative sites and environments in Europe and Canada, and it is concluded that oilseed rape MS8, RF3 and MS8 x RF3 is agronomically and phenotypically equivalent to the conventional counterpart, except for the newly expressed barnase, barstar and PAT proteins.

The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glufosinate herbicide do not alter the phenotypic and agronomic characteristics of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape varieties.

Environmental risk

Considering the scope of the notification C/BE/96/01, excluding cultivation purposes, the environmental risk assessment is limited to exposure through accidental spillage of viable seeds of MS8, RF3 and MS8 x RF3 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.

Accidental spillage and loss of viable seeds of MS8, RF3 and MS8 x RF3 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 MS8, RF3 and MS8 x RF3 cannot be excluded. Feral oilseed rape MS8, RF3 and MS8 x RF3 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 in Norway.

There is no evidence that the herbicide tolerant trait results in enhanced fitness, persistence or invasiveness of oilseed rape MS8, RF3 and MS8 x RF3, or hybridizing wild relatives, compared to conventional oilseed rape varieties, unless the plants are exposed to herbicides with the active substance glufosinate ammonium. Apart from the glufosinate 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.

Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 for reasons of reproductive toxicity.

Overall conclusion
The VKM GMO Panel concludes that oilseed rape MS8, RF3 and MS8xRF3 are unlikely to have any adverse effect on the environment in Norway in the context of its intended usage.

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Appendix 1

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

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

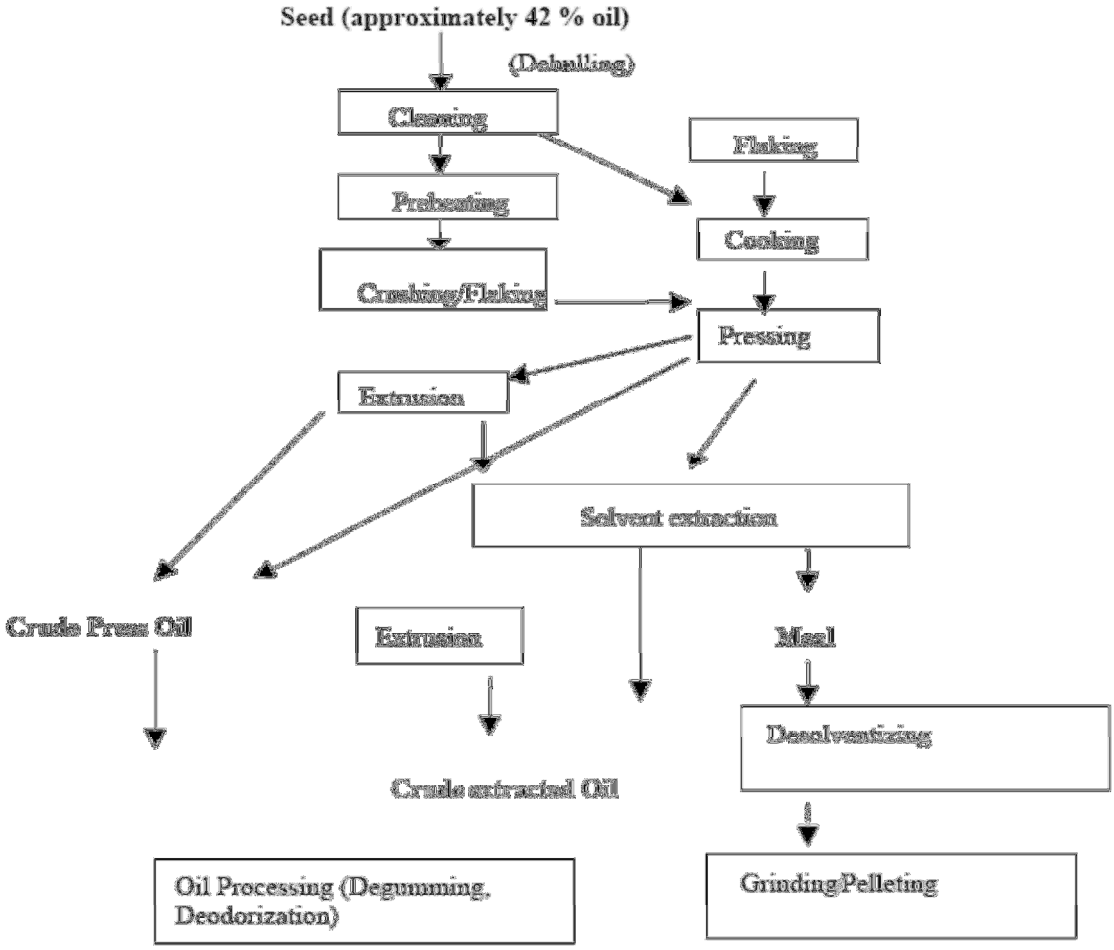

Figure 1. **Schematic illustration of the processing of low erucic acid rapeseed meal and low erucic acid rapeseed oil (OECD 2001).**

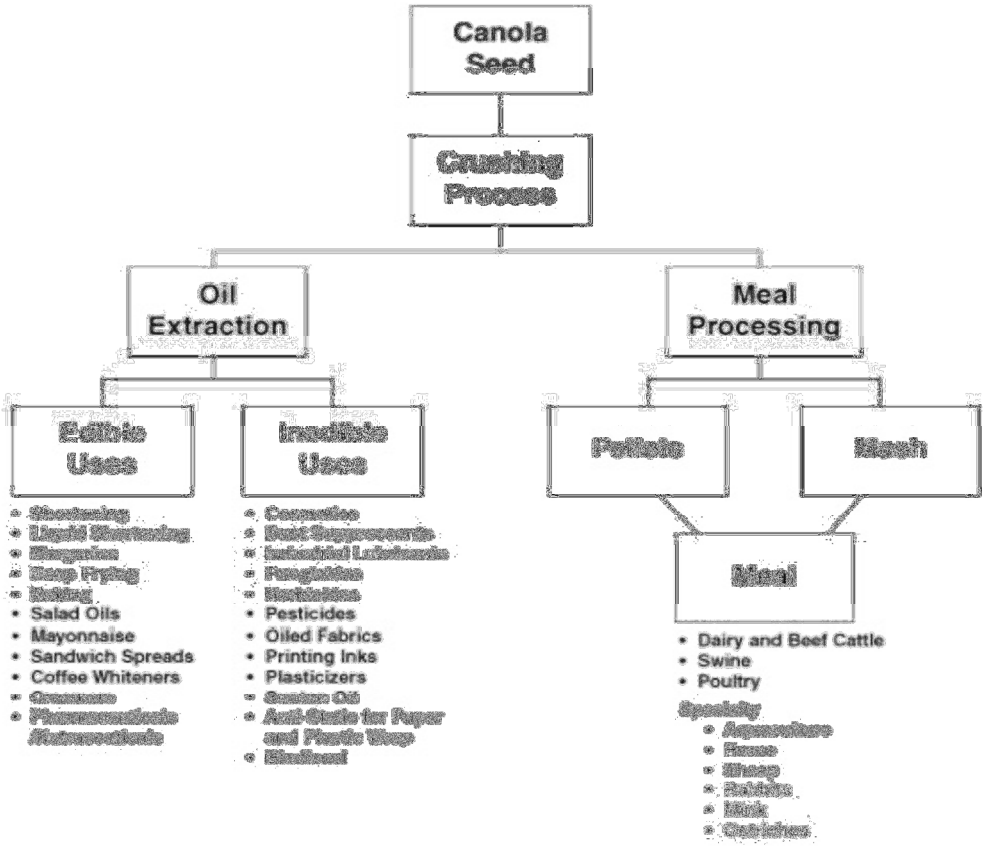

Figure 2 **Areas of application and products from processing of rapeseed (Canola Council of Canada 2005).**

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 % F_1-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.

F_1-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 F_1-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 BC_3-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 (F_1-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 F_1-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 BC_3-hybrids and BC_4-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 F_1-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 F_1-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 % F_1-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;

Chévre et al. 1997, 1998, 2000; Rieger et al. 2001; Warwick et al. 2003). Depending on genotype, Chévre 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 F_1-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 F_1-hybrids are sterile or have low fertility, and there is no form of seed dormancy.