Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food/feed and environmental risk assessment of genetically modified glufosinate-tolerant 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

Date: 28 March 2014

Doc. no.: 13/316 – final

ISBN: 978-82-8259-104-1

VKM Report 2014: 29

Contributors

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

Acknowledgements

Monica Sanden, The National Institute of Nutrition and Seafood Research, is acknowledged for her valuable work on this opinion.

Assessed by

Panel on Genetically Modified Organisms
Åshild Andreassen (Chair), Per Brandtzæg, Hilde-Gunn Hoen-Sorteberg, Askild Holck, Olavi Junttila, Heidi Sjursen Konestabo, Richard Meadow, Kåre M. Nielsen, Rose Vikse

Scientific coordinators from the secretariat
Merethe Aasmo Finne, Anne Marte Jevnaker, Arne Mikalsen, Ville Erling Sipinen

Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency (former Norwegian Directorate for Nature Management) has requested the Norwegian Food Safety Authority (NFSA) to give final opinions on 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 within the Authority's sectoral responsibility. The Norwegian Food Safety Authority has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested the Norwegian Scientific Committee for Food Safety (VKM) to carry out scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorized in the European Union. 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 Agency 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 food/feed and environmental risk assessment was commissioned by the Norwegian Environment Agency 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 risk assessments of genetically modified plants and new scientific literature, the VKM GMO Panel has decided to deliver an updated food, feed and 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 x RF3 for food containing or consisting of, and food produced from or containing ingredients produced from MS8 x RF3 (with the exception of processed oil) (EFSA/GMO/BE/2010/81) have also been submitted by the VKM GMO Panel (VKM 2012, unpublished).

The 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/2010/81, 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 2010a) and the selection of comparators for the risk assessment of GM plants (EFSA 2011b).

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 compositional assessment, food/feed safety assessment, comparative assessment of agronomic and phenotypic characteristics, unintended effects on plant fitness and potential for horizontal and vertical gene transfer.

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 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 MS8 x RF3, and the desired traits are stably inherited over generations.

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 MS8 x RF3 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 compositionally, agronomically and phenotypically equivalent to the conventional counterpart, except for the newly expressed barnase, barstar and PAT proteins.

In the Canadian field trials, however, compositional and phenotypic characteristics of oilseed rape MS8, RF3 and MS8 x RF3 were compared to null-segregant comparators. As negative segregants are derived from a GM organism, the VKM GMO Panel does not consider them appropriate conventional counterparts with a history of safe use. Data obtained from field trials with negative segregants were considered as supplementary information for the RA.

Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of oilseed rape MS8 and RF3 to produce the hybrid MS8 x RF3 does not result in interactions that cause compositional, agronomic and phenotypic changes that would raise safety concerns.

Food and feed risk assessment

Whole food feeding studies in broilers have not indicated any adverse health effects of oilseed rape MS8 x RF3. These studies also indicate that oilseed rape MS8 x RF3 is nutritionally equivalent to conventional oilseed rape. The PAT protein do not show sequence resemblance to other known toxins or IgE allergens, nor has PAT been reported to cause IgE mediated allergic reactions.

Based on the current knowledge, the VKM GMO Panel concludes that oilseed rape MS8 x RF3 is nutritionally equivalent to conventional oilseed rape varieties, and that it is unlikely that the newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from oilseed rape MS8 x RF3 compared to conventional oilseed rape.

Environmental risk assessment

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
Based on current knowledge, the VKM GMO Panel has not identified toxic, allergenic or altered nutritional properties of oilseed rape MS8, RF3 and MS8 x RF3 or its processed products compared to conventional oilseed rape.

The VKM GMO Panel likewise concludes that oilseed rape MS8, RF3 and MS8 x RF3 are unlikely to have any adverse effect on the environment and agriculture 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, EFSA/GMO/RX/MS8/RF3, EFSA/GMO/BE/2010/81, glufosinate-tolerant, *bar, barnase, barstar,* PAT protein, food and feed risk assessment, 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 har Miljødirektoratet (tidligere Direktoratet for Naturforvalting) bedt Mattilsynet om vurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18 som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. På den bakgrunnen har Mattilsynet, i brev av 13. februar 2013 (ref. 2012/150202), bedt Vitenskapskomiteen for mattrygghet (VKM) om å utarbeide endelige vitenskapelige risikovurderinger av 39 GMOer og avledete produkter som inneholder eller består av genmodifiserte organismer, innen Mattilsynets sektoransvar. VKM er bedt om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelig risikovurdering. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige risikovurderingene 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 i EU under direktiv 2001/18 26. mars 2007 (Kommisjonsbeslutning 2007/232/EC). I tillegg ble prosesserte oljer fra MS8 og RF3, og MS8 x RF3 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/MS8/RF3). Videre ble det i 2010 fremmet en søknad om godkjenning av MS8 x 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 x RF3. I henhold til søker var bakgrunnen for søknaden å ivareta/dekke opp for utilsiktet innblanding av sporforurensinger av MS8 x RF3 i matkjeden.

Rapslinjene MS8 x 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 risikovurdering av MS8, RF3 og MS8 x RF3. 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, 2011 a, 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 (MS8 x RF3). 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 (published)).

Komparative analyser
Data fra feltforsøk i Canada og Europa indikerer ernæringsmessig, agronomisk og fenotypisk ekvivalens mellom de transgene rapslinjene MS8, RF3 and MS8 x RF3 og umodifisert kontroll, med unntak av de introduserte proteinene barnase, barstar og PAT.

Det bemerkes imidlertid at søker har benyttet negative segreganter som komparator i de kanadiske feltforsøkene. Negative segreganter er avledet fra genmodifiserte organismer og VKMs faggruppe for GMO anser ikke at disse tilfredstiller kravene til konvensjonelle komparatorer med «history of safe use». Data fra feltforsøk med negative segreganter kan imidlertid være nyttig tilleggsinformasjon i risikovurderinger av GMO.

Basert på tilgjengelig data, konkluderer VKM med at konvensjonelle kryssinger mellom de genmodifiserte oljerapslinjene MS8 og RF3 for å danne hybriden MS8 x RF3, ikke resulterer i interaksjoner som medfører endringer i ernæringsmessige, agronomiske og fenotypiske karakterer.

Helserisiko
Ingen negative helseeffekter relatert til rapslinjen MS8 x RF3 ble rapportert fra fôringsstudie med hel mat utført på broilere. Bioinformatikk-analyser viser ingen likheter mellom de introduserte proteinene og kjente toksiner eller allergener. Det er derfor ikke grunnlag for å anta at egenskapene til prosesserte produkter fra rapslinjen MS8 x RF3 vil være forskjellige fra prosesserte produkter basert på konvensjonelle rapssorter.

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 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 har, ut fra dagens kunnskap, ikke identifisert toksiske, allergent eller endrede ernæringsmessige egenskaper hos oljerapslinjene MS8, RF3 og MS8xRF3 sammenlignet med konvensjonell raps. Faggruppen finner det lite trolig at den omsøkte bruken av oljerapslinjene MS8, RF3 og MS8 x RF3 vil medføre endret risiko for miljø og landbruk 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
ENTRY	Plant + herbicide treatment (i.e. transgenic oilseed rape MS8, RF3, and MS8xRF3 treated with glufosinate ammonium, or the non-transgenic counterparts treated with conventional herbicides)
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	glufosinate-ammonium
GLP	Good Laboratory Practices
Glufosinate-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.

Table of contents

Background

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 risk assessment was commissioned by the Norwegian Environment Agency 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 food/feed and environmental risk assessments of genetically modified plants and new scientific literature, the VKM GMO Panel has decided to deliver an updated food/feed and 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 MS8 x RF3 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 MS8 x RF3, 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 MS8 x RF3 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 MS8 x RF3. 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, not published).

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

The Norwegian Environment Agency has the overall responsibility for processing applications for the deliberate release of genetically modified organisms (GMOs). This entails inter alia coordinating the approval process, and to make a holistic assessment and recommendation to the Ministry of the Environment regarding the final authorization process in Norway. The Directorate is responsible for assessing environmental risks on the deliberate release of GMOs, and to assess the product's impact on sustainability, benefit to society and ethics under the Gene Technology Act.

The Norwegian Food Safety Authority (NFSA) is responsible for assessing risks to human and animal health on deliberate release of GMOs pursuant to the Gene Technology Act and the Food Safety Act. In addition, the NFSA administers the legislation for processed products derived from GMO and the impact assessment on Norwegian agriculture according to sector legislation.

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested the Norwegian Food Safety Authority to give final opinions on 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 within the Authority's sectoral responsibility. The request covers scope(s) relevant to the Gene Technology Act.

The Norwegian Food Safety Authority has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested the Norwegian Scientific Committee for Food Safety (VKM) to carry out final scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorized in the European Union.

The assignment from NFSA includes food and feed safety assessments of genetically modified organisms and their derivatives, including processed non-germinating products, intended for use as or in food or feed.

In the case of submissions regarding genetically modified plants (GMPs) that are relevant for cultivation in Norway, VKM is also requested to evaluate the potential risks of GMPs to the Norwegian agriculture and/or environment. Depending on the intended use of the GMP(s), the environmental risk assessment should be related to import, transport, refinement, processing and cultivation. If the submission seeks to approve the GMP(s) for cultivation, VKM is requested to evaluate the potential environmental risks of implementing the plant(s) in Norwegian agriculture compared to existing varieties (e.g. consequences of new genetic traits, altered use of pesticides and tillage). The assignment covers both direct and secondary effects of altered cultivating practices.

VKM is further requested to assess risks concerning coexistence of cultivars. The assessment should cover potential gene flow from the GMP(s) to conventional and organic crops as well as to compatible wild relatives in semi-natural or natural habitats. The potential for establishment of volunteer populations within the agricultural production systems should also be considered. VKM is also requested to evaluate relevant segregation measures to secure coexistence during agricultural operations up to harvesting. Post-harvest operations, transport, storage are not included in the assignment.

Evaluations of suggested measures for post-market environmental monitoring provided by the applicant, case-specific monitoring and general surveillance, are not covered by the assignment from the Norwegian Food Safety Authority.

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 2010a), 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 food/feed and 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/2010/81, 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 MS8 x RF3 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. MS8 x RF3contains 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 x 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

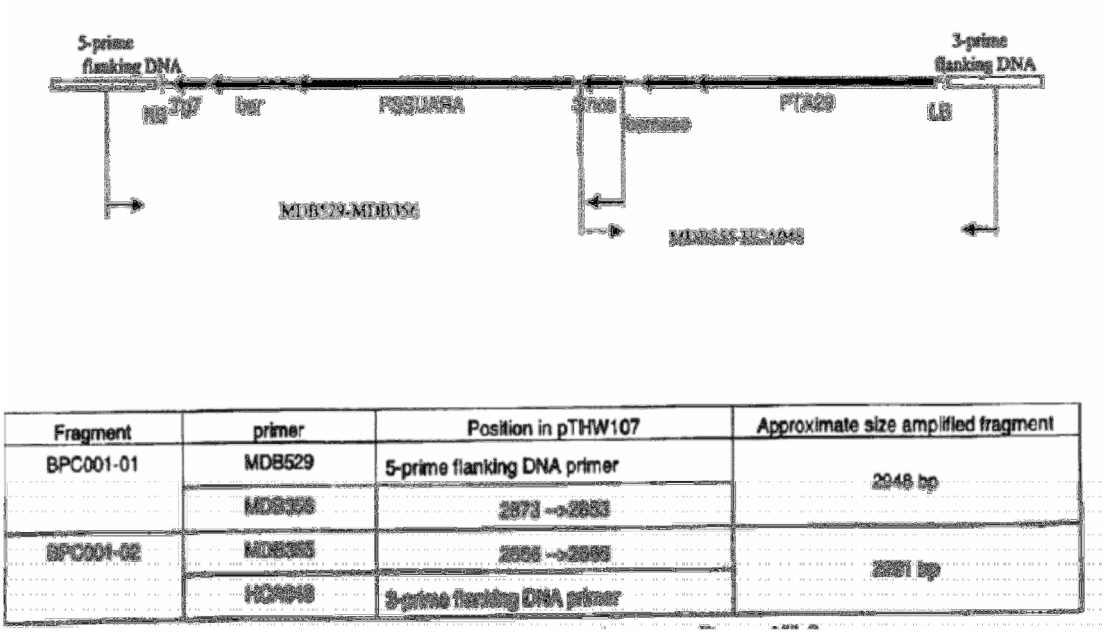
MS8 oilseed rape (male sterile line)
Southern blot analysis of MS8 oilseed rape genomic DNA has been carried out with a set of Southern probes spanning the entire length of the T-DNA region of plasmid pTHW107 in combination with different restriction digests. The applicant concludes that these Southern 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*.



Fragment	primer	Position in pTHW107	Approximate size amplified fragment
BPC001-01	MDB529	5-prime flanking DNA primer	2048 bp
	MDB356	2871 –>2853	
BPC001-02	MDB355	2856 –>2868	2881 bp
	HCA048	3-prime flanking DNA primer	

Figure 1. Amplification strategy – male sterile line MS8.

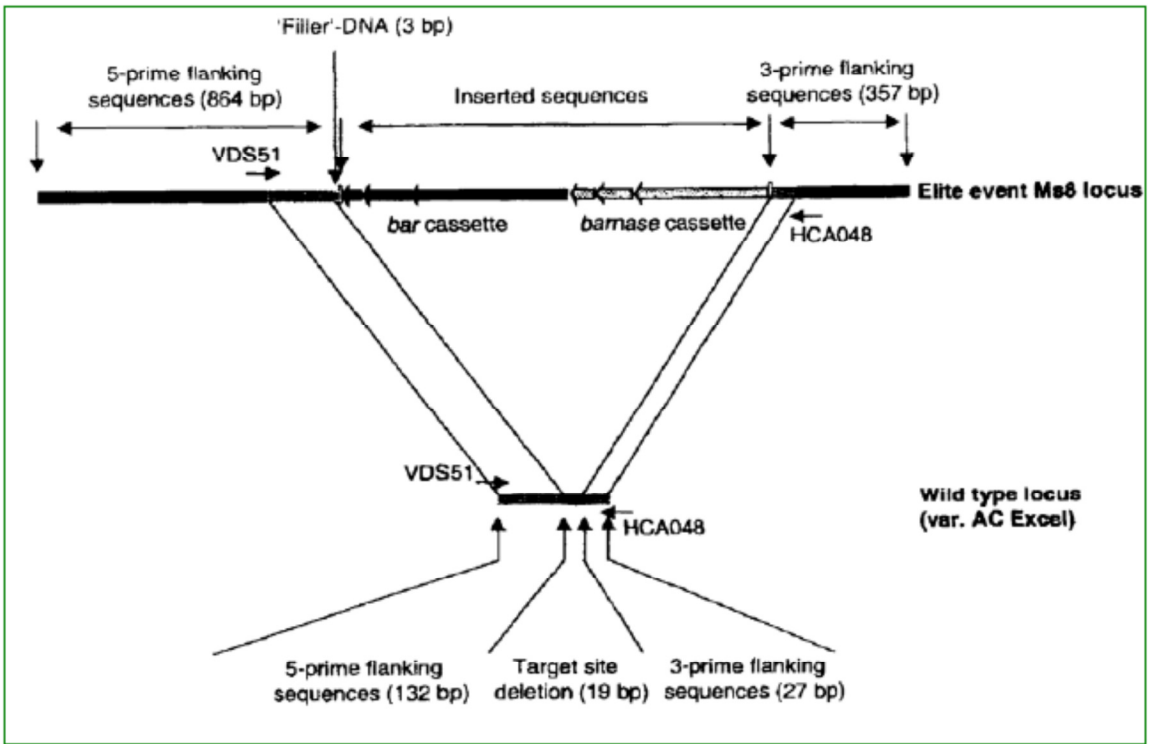


Figure 2. Physical map of the insert of event MS8 and schematic representation of the alignment of the MS8 transgene locus and the wild type locus.

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.

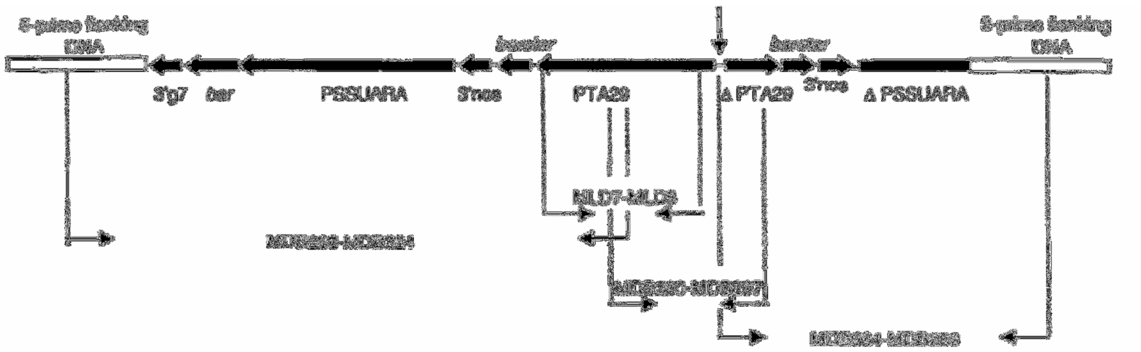
A detailed description of the RF3 molecular characterization has been provided as additional information to EFSA in January 2009 in the frame of application EFSA-GMO-RX-MS8-RF3.

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*.



Fragment	primer	Position in pTHW118	Approximate size amplified fragment
BPC002-01	MDB268	5-prime flanking DNA primer	3400 bp
	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]
	MDB297	3453→3475	
BPC002-04	MDB534	3656→3676	3255 bp
	[illegible]	[illegible]	

Figure 3. Amplification strategy, fertility restorer RF3.

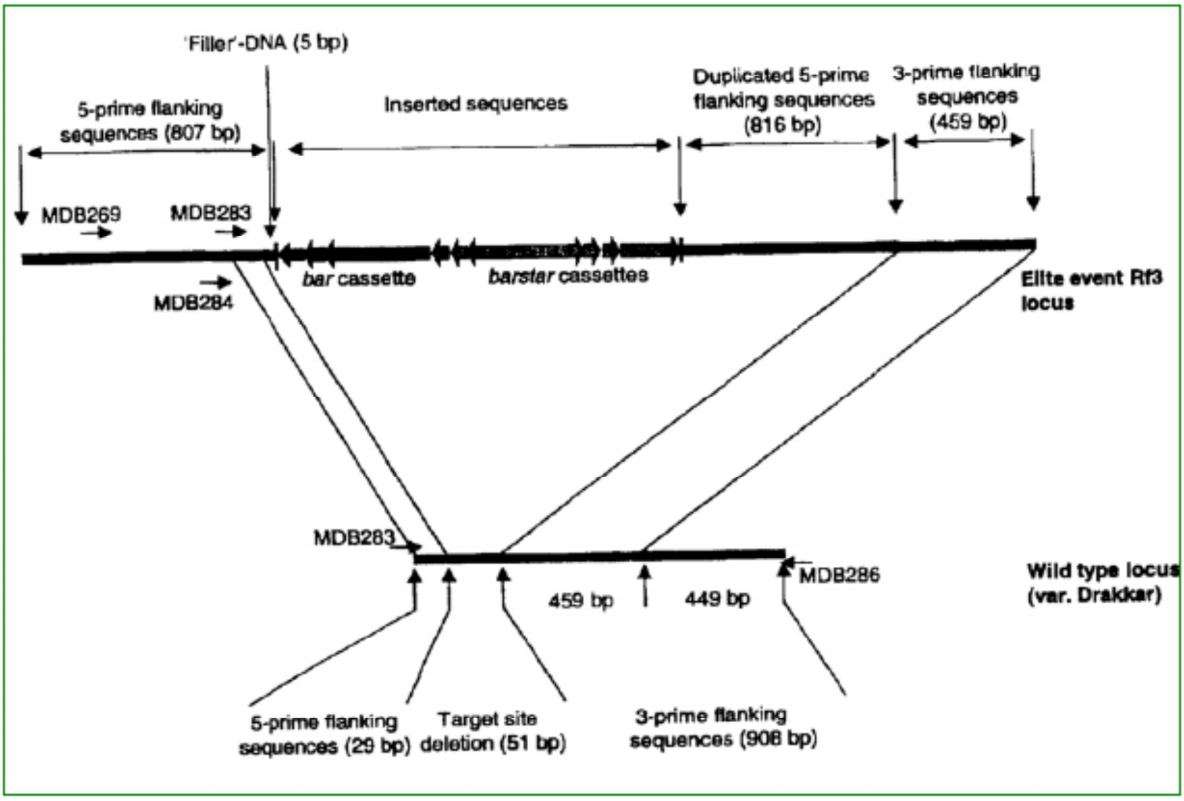


Figure 4. Physical map of the insert of event RF3 and schematic representation of the alignment of the RF3 transgene locus and the wild type locus.

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

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 MS8 x RF3	1.6-3.2 3.2-6.4 3.2	- ND -	ND - -
Mature leaf	MS8 RF3 MS8 x RF3	3.2 3.2-6.4 3.2-6.4	- ND -	ND - -
Root	MS8 RF3 MS8 x RF3	- 0.2 0.2	- ND -	ND - -
Flower bud	MS8 RF3 MS8 x RF3	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	Not analysed -	- ND	Not analysed -

	MS8 x RF3	-	-	-
Dry seed	MS8 RF3 MS8 x RF3	- - -	- ND -	ND - -
Immature seed	MS8 RF3 MS8 x RF3	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 MS8 x RF3 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 RF3 MS8 x RF3 Wt	- Not analysed - -	Not analysed - - -	Not analysed Not analysed - -	+ + + -
Root	MS8 RF3 MS8 x RF3 Wt	- Not analysed - -	Not analysed - - -	Not analysed Not analysed - -	+ + + -
Mature leaf	MS8 RF3 MS8 x RF3 Wt	- Not analysed - -	Not analysed - - -	- Not analysed - -	+ + + -

Flower bud	MS8 RF3 MS8 x RF3 Wt	- Not analysed +* -	Not analysed + +* -	- Not analysed +* -	+ + + -
Pollen	RF3 MS8 x RF3 Wt	Not analysed - -	- - -	Not analysed - -	+ + -
Dry seed	MS8 RF3 MS8 x RF3 Wt	- Not analysed - -	Not analysed - - -	- Not analysed - -	+ + + -

+: 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 x 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 x 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 x RF3	0.5044	14.78	0.112	0.008	0.22	0.0008	+
SOSR	F2	MS8 x 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 MS8 x RF3 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 MS8 x RF3, 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 MS8 x RF3. 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 x 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 x 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 MS8 x RF3 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 MS8 x RF3, and the desired traits are stably inherited over generations.

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 MS8 x RF3 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 published scientific opinions on MS8, RF3 and MS8 x RF3 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 oilseeds grown in Norway 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(s) and production of material for the compositional assessment

The transgenic oilseed rape lines MS8, RF3 and derived hybrid MS8 x RF3 have been tested in field trials 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 compositional, agronomic and phenotypic data on oilseed rape MS8 x RF3 have been previously evaluated by the VKM GMO Panel in 2008 (VKM 2008). A food, feed and environmental risk assessment on the application MS8, RF3 and MS8 x RF3 (EFSA/GMO/BE/2010/81) has also been evaluated by the VKM GMO Panel in 2012 (VKM 2012, unpublished).

4.1.1 Experimental design and 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.

Further, to the assessment previously conducted in the frame of notification C/BE/96/01 and application EFSA-GMO-RX-MS8-RF3 additional data are provided by the applicant. The applicant provided, in the frame of the application EFSA/GMO/BE/2010/81, compositional data from additional field trials performed at five locations in Canada during the 2008 growing season. Oilseed rape MS8 and RF3, their non-transgenic counterparts and commercial oilseed rape hybrids of MS8 x RF3 were field tested by Bayer CropScience. In these field trials, oilseed rapes were treated with the target herbicides. The comparators used were negative segregants that had been isolated, after several stages of backcrossing and selfing of the progeny of the initial transformant, from rapeseed that was essentially homozygous for the insert in MS8 x RF3. The VKM´s as well as EFSA´s GMO Panels do not consider negative segregants derived from GM organisms as appropriate conventional counterparts with a history of safe use (EFSA 2006; EFSA 2011a). Data obtained from field trials with negative segregants are considered as supplementary information only.

The field trials were designed as randomised complete block designs with four replicates and were compared with comparators consisting of negative segregants from these oilseed rape events untreated with the target herbicide.

For each analyzed component, mean values, standard deviations, minimum and maximum were calculated per site and over all sites. For each component the data were analysed with analysis of variance (ANOVA) methods using a model with fixed factors ENTRY (plant + treatement i.e. glufosinate treated MS8, glufosinate treated RF3, glufosinat treated MS8xRF3, or conventionaly treated non-transgenic counterparts with the same genetic hybrid background) and SITE (for location) as well as their interaction term. Based on the ANOVA model entry differences were estimated and presented together with 95% confidence intervals. Entry comparisons are only valid in cases of no entry*site interactions. In cases when interaction between the factors ENTRY and SITE were detected in the over all analyses (p-value ANOVA E*S < 0.05), the results of the by-site analyses are presented. For each component the analysis was performed with ANOVA methods including the factor ENTRY, followed by t-tests. Based on the ANOVA, entry differences and 95% confidence intervals were estimated.

4.2 Compositional analysis

4.2.1 Compositional assessment of application EFSA/GMO/RX/MS8/RF3

In total 144 samples from 12 sites taken over two years as part of 2 field trial studies were analysed for a maximum of 70 components. Ash, water, fiber (ADF, NDF), proteins, carbohydrates, amino acids, fatty acids, minerals (phosphorus, iron, potassium, calcium, copper, magnesium, manganese, sodium, zinc), vitamins (alfa-, beta-, gamma- and deltatocopherol, total tocopherol (vitamin E)) and anti-nutrients have been analyzed in grain of oilseed rape MS8 x RF3. Protein and amino acids have also been analyzed in oil fractions and refined oil. Proteins and amino acids were not detected in the refined oil. Several of the components listed in in OECD consensus documents have not been analyzed in grain and feed products.

The separation of the oil fraction from the rapeseed results in an increase of the content for most of the components of the meal matrix. Therefore protein, ash (sum of the minerals), total carbohydrates and fibre contents are higher in rapeseed meal compared to the seeds. For most minerals the measured values fall slightly short of the range reported from literature. In case of the trace elements, iron, manganese, copper and zinc, this might be due to the fact that reference data are only available for rapeseed meal, and not for the raw product seed. For calcium some differences are noticed (Table 2 – appendix 2).

Proximates

The components selected for compositional analyses of proximates are moisture, protein, fat, ash, carbohydrates and fibre. In all sites the components are homogenous in the reference group (non-transgenic, not Liberty®sprayed), that is, the coefficient of variance is less than 20% - with exception of acid detergent fibre (ADF) and neutral detergent fibre (NDF). Equivalence of the three treatments can be assumed for the components moisture, total fat, protein, ash and total carbohydrates. The defined range of 20% as standard equivalence criterion corresponds to the natural variation for each proximate compound in the non-transgenic material. A range of 20% was considered acceptable and should meet most of the natural variation ranges for the measured compounds (TemaNord 1998).

For the components ADF and NDF the results are ambiguous within the sites (Table 1). Differences in the transgenic samples compared to the non-transgenic control samples for ADF can be noticed, but the maximum deviation in % of the control mean is not exceeding 35%. For NDF minor findings in the transgenic samples are found. The maximum deviation is calculated with 41% of the control mean. Even the comparison between the two transgenic sample groups shows very often no bio-equivalence. In the overall comparisons (no treatment * site interaction; p-value > 0.05) equivalence of the three treatments can be assumed for the two fibres.

Further, most values are in agreement with the range built from literature data. For the components moisture, total fat, protein, ash and total carbohydrates, no statistical significant differences were found (Table 1 – appendix 2). The compositional assessments are performed using the principles and analytes outlined in the OECD consensus document for low erucic acid oilseed rape composition (OECD 2011).

Minerals

Sodium was measurable in less than one third of all sites. For this component only the overall analysis was done, in which equivalence could be stated for the three treatments. The coefficient of variance is less than 20% in all sites in the reference group (non-transgenic, not Liberty® sprayed) for calcium, phosphorus, potassium, magnesium and manganese. For the components iron and zinc the coefficient of variance is larger than 20% only in one site each. Further, more variation is found for the component copper. The natural variation for copper in the non-transgenic material exceeds the defined range of 20% for the standard equivalence criterion. As a consequence, many comparisons result in no bioequivalence for copper between the different treatments.

Since the copper content measured in the transgenic samples is exceeding as well as falling short of the 20% range built from the control mean, no unifrom tendency of minor or major findings is stated. The copper results of the single sites show that in some replicates of the three different treatments extraordinary high copper contents are found. According to the applicant, the reason for this could be a contamination of the samples with copper in the time between harvest and analytical test in the laboratory. An analytical error can be excluded, since in some samples copper was double tested in the course of quality control requirements, and the high copper contents in the samples were confirmed. Therefore, a second statistical analysis was performed omitting the extremely high copper values. In this second statistical analysis equivalence of the three treatments was stated in most of the sites. In the overall comparison (p-value of interaction > 0.05) equivalence of the three treatments was assumed for copper, too.

All minerals, except cobalt, selenium and iodine, were measured according to OECD consensus document (OECD 2002). The main mineral elements in canola are calcium, phosphorus, magnesium, potassium and sodium. Trace elements include iron, manganese, zinc, and copper. For most minerals the measured values fall slightly short of the range reported from literature (Table 2 – appendix 2). In case of the trace elements, iron, manganese, copper and zinc, this might be due to the fact that reference data are only available for rapeseed meal, and not for the raw product seed. The separation

of the oil fraction from the rapeseed results in an increase of the content for all other components of the meal matrix

Tocopherols
According to the applicant vitamin E (α-tocopherol) is a nutrient component in oilseed rape that is known to be important in maintaining the oxidative stability of rapeseed oil. Levels in oilseed rape seed are reported to vary from 71.1 to 108.4 mg/kg dwt based on environment and germplasm. For human nutrition, it is important to assess both vitamin E and vitamin K_1 content of the oil (OECD 2011). OECD recommends that vitamin E and vitamin K_1 are analyzed in seed and/or oil and meal (OECD 2011).

The applicant has analyzed for tocopherols (vitamin Es) in rapeseed (Table 3 – appendix 2). Vitamin K has not been analyzed. The components beta and delta tocopherols could not be quantified in all seed samples from all sites because the results were below LOQ. For these two tocopherols bio-equivalence is stated by the applicant, because the non-transgenic and the transgenic samples had levels < 1.00 mg/100g dm. According to the applicant overall comparisons (p-value of interaction > 0.05) of the three treatments concludes that equivalence can be assumed for alpha and gamma tocopherols.

Anti-nutrients
According to OECD (2001, 2011), oilseed rape contains two potential toxicants, erucic acid and glucosinolates, and the anti-nutrient components, phytic acid and sinapine. Because erucic acid has been historically associated with cardiopathic potential in animal species, the Codex Standard for Named Vegetable Oils (Codex Alimentarius 2005) specifies that erucic acid in rapeseed oil for human consumption cannot exceed more than 2% of total fatty acids.

Phytic acid, alkenyl glucosinolate, aromatic glucosinolate, indolyl glucosinolate and total glucosinolate were analyzed. Sinapine was not analyzed. Sinapine has several undesirable properties as a constituent in animal feeds. It is a bitter tasting compound, making it less palatable to animals, while its presence in the diet of certain brown egg laying hens at levels exceeding 1 g/kg leads to a fishy odour or taste in the eggs (OECD 2011).

A statistical analysis of variance (ANOVA) of glucosinolate data as provided by the applicant showed statistically significant differences in the contents of alkenyl glucosinolates (18 %) and total glucosinolates (16 %) between the GM oilseed rape and its non-GM comparator Drakkar. However, these differences were not considered biologically relevant given the reported natural variations in these compounds in oilseed rape (OECD 2011). The maximum level of glucosinolate content is set to 25 µmol/g by the European Commission for certified seed of "double zero" varieties listed in the Common Catalogue of Varieties of Agricultural Plant Species (EC 1999). All treatment groups groups had glucosinolate levels below this limit.

The maximum absolute difference between mean values of transgenic and nontransgenic samples for the alkenyl glucosinolate content is 4.0 umol/g and for the total glucosinolate content 4,1 umol/g. But even between the two transgenic sample groups a difference between the mean values of about maximum 2,6 umol/g are calculated. The average difference between mean values of transgenic and non-transgenic samples summarized from all sites is 2.1umol/g for the alkenyl glucosinolate content and 2,2umol/g for the total glucosinolate content.

The mean total glucosinolate content for all rapeseed samples, non-transgenic and transgenic, is below the different national thresholds of 30umol/g in air-dried seed (Canada) or 25 umol/g in air-dried seed (Europe). The glucosinolates themselves are not the anti-nutritional compound but their hydrolysis products. So an increase of 15% in the total glucosinolate content does not lead to an increase of the anti-nutritional hydrolysis products per se.

Rapeseeds are not eaten as whole, not further processed, agricultural commodity by humans and animals. The main oilseed rape products that go into the food and feed chain are food grade oil and rapeseed meal. The oil processing, especially the seed cooking and conditioning (heating of the flaked seeds) is the step that inactivates the enzym myrosinase.

During the solvent extraction and drying of the meal a complete myrosinase inactivation is achieved. So the increase of 15% in the glucosinolate content in the seeds do not lead to higher hydrolysis product in the oil or meal, because the enzyme myrosinase is destroyed (Mag 2001). Summarising these three statements the increase of 15% in the alkenyl glucosinolate content found over all sites has no nutritional relevance for humans and animals consuming oil products or animals fed rapeseed meal derived of SL OSR event Ms8Rf3 seeds.

The content of aromatic glucosinolate was below the limit of quantification in a number of samples. For the equivalence analysis only those sites were considered, in which more than one third of the values were measurable. Coefficients of variance of more than 20% occurred in the reference group (non-transgenic, not Liberty®treated) only for a small number of sites and components.

Equivalence of the three treatments can be assumed for phytic acid. For this anti-nutrient equivalence is stated in most of the site-by-site comparisons and in the overall sites comparisons (p-value of interaction > 0.05).

The results from the anti-nutrients analyses and their comparison with data from literature are presented in the Table 4 – appendix 2. Literature values were only found for the total glucosinolate content in OSR seeds (OECD 2001). Values measured for the non-transgenic control and the transgenic samples are inside the range for total glucosinolate from OSR varieties currently on the market. The SL OSR event MS8xRF3 might have statistically higher alkenyl and total glucosinolate values compared to its non-transgenic counterpart, but not, if compared to other commercial OSR varieties (CO-OP recommendation data).

Amino acids

In all sites, all total amino acids were homogenous in the reference group (non-transgenic, not Liberty treated). The coefficient of variance is less than 20% in all cases. Equivalence of the three treatments can be assumed for all amino acids and was stated in most of the comparisons within and over all sites.

Essential amino acids were analyzed according to the OECD consensus document for LEAR (OECD 2011). The measured total amino acid values were in compliance with the reported OECD reference ranges (Table 5 – appendix 2). The statistical differences that were found for aspartic acid and glutamic acid were $p \leq 0.05$. Reference data for these two amino acids were only found for rapeseed meal. This commodity has a total protein content that is twice as large as the raw seeds (seeds 18,7-26,0% dm protein; meal 32,0-40,4% dm protein). Consequently the reference values for the two amino acids were also two times higher than the determined contents in the seed matrix.

Fatty acids

Values for the following fatty acids were found to be below the limit of detection in all sites and all samples: C8:0 Octanoic (Caprylic), C10:0 Decanoic (Capric), C12:0 Duodecanoic (Lauric), C14:0 Tetradecanoic (Myristic), C14:1 Tetradecenoic (Myristoleic), C15:0 Pentadecanoic, C15:1 Pentadecenoic, C17:0 Heptadecanoic (Margaric), gamma C18:3 Octadecatrienoic (gamma Linolenic), C20:2 Eicosadienoic, C20:3 Eicosatrienoic, C20:4 Eicosatetraenoic (Arachidonic), C20:5 Eicosapentaenoic, and C22:1 Docosenoic (Erucic). The data from these components were not analysed further. For these fatty acids equivalence can be assumed between the non-transgenic and transgenic samples. This is especially important for the fatty acid erucic acid (C22:1), which belongs to the components with anti-nutritional features in rapeseeds. Values for C17:1 (Heptadecenoic) was below the limit of detection (0.05) or equal 0.05 in all sites and all samples. Only the overall analysis was done for this component. For the equivalence analysis of the fatty acids C22:5 (Docosapentaenoic),

C22:6 (Docosahexaenoic) and C24:0 (Lignoceric) only sites in which more than one third of the values were measurable.were considered.

The major fatty acids in oilseed rape are oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and palmitic acid (C16:0). Equivalence is stated in the site-by-site analysis between the non-transgenic, the transgenic not sprayed and the transgenic Liberty sprayed for most total fatty acids (Table 6 and 7 – appendix 2).

The fatty acid composition was measured according to OECD consensus documents for low erucic acid oilseed rape (LEAR) (OECD 2011). Measurement from both glufosinate treated and conventional herbicide treated transgenic oilseed rape was reported. The measured fatty acids in the dossier are reported in % based on the seed dry weight. Since the most reference guides show the fatty acid profile of rapeseed oil in % relative of the total amounts of fatty acids, the measured values has been converted to % relative fatty acid values by the applicant, in order to correspond to the data reported from literature.

4.2.3 Compositional assessment of application EFSA/GMO/BE/2010/81

The scope of the application has been selected in order to cover accidental, unintentional presence of traces of MS8 x RF3 oilseed rape seed in food. Comparative assessment has been carried out with MS8, RF3, MS8 x RF3 and MS8 x RT73 oilseed rape and a nontransgenic negative segregant with the same genetic background. In addition, composition data derived from publicly available literature references, including data from non-GM oilseed rape varieties have been used as the baseline. Since the scope of application EFSA/GMO/BE/2010/81 cover MS8 x RF3, compositional assessments of RT73 and MS8 x RT73 have been omitted.

In addition to previously submitted data from field trials at 12 different locations in Europe (Belgium) performed in the growing seasons 2001 and 2002 (EFSA-GMO-RX-MS8-RF3), supplementary data for comparative assessment of compositional and nutritional equivalence of MS8 x RF3 oilseed rape were generated from oilseed rape seed collected during a field trial carried out at five locations in Canada during the 2008 growing season (Oberdörfer 2009 M-352327-01-1; Oberdörfer 2009 M-357182-01-1).

The mean values and minimum/maximum ranges of compositional data for MS8, RF3, MS8xRF3, their non-transgenic counterparts with the same genetic hybrid background and reference ranges reported from literature have been collected. In addition, composition data derived from publicly available literature references, including data from non-GM oilseed rape varieties, have been used as the baseline.

Proximates and fibers
The components selected for compositional analyses of proximates are moisture, protein, fat, ash, carbohydrates and fibre. No significant differences between ENTRY (glufosinat treated MS8, glufosinate treated RF3, glufosinat treated MS8xRF3, or conventionaly treated non-transgenic counterparts with the same genetic background) mean values over all sites ($p>0.05$) were found for all proximate and fibre compounds (Table 8 – appendix 2).

Minerals and tocopherols
All minerals, except cobalt, selenium and iodine, were measured according to OECD consensus document (OECD 2011). The major mineral elements in canola are calcium, phosphorus, magnesium, potassium and sodium. Trace elements include iron, manganese, zinc, and copper.

No significant differences between ENTRY mean values over all sites ($p> 0.05$) were found for most minerals and tocopherols (Table 9 – appendix 2). According to the applicant, all mean values for the

minerals sodium, copper and zinc are lower than the reference values. However, according to the OECD consensus document on low erucic rapeseed these three minerals are within the OECD-reference range (OECD 2011). There is only minor difference in alpha-tocopherol between RF3 and MS8, and RF3 and MS8 x RF3. For the tocopherol isomers with a high biological activity, equivalence between the entries was proven.

Total amino acids
Essential amino acids were analyzed according to the OECD consensus document for LEAR (OECD 2011). No significant differences between ENTRY mean values over all sites ($p > 0.05$) were found for all total amino acids (Table 10 – appendix 2).

Anti-nutrients
According to OECD (2001, 2011), oilseed rape contains two potential toxicants, erucic acid and glucosinolates, and the anti-nutrient components, phytic acid and sinapine. No significant differences between ENTRY mean values over all sites ($p>0.05$) were found for phytic acid and methylthiobutenyl glucosinolate (MSGL) (Table 11 – appendix 2). Total glucosinolate contents are all below the safety threshold of 30µmol/g.

Total fatty acids
The fatty acid composition was measured according to OECD consensus documents for low erucic acid oilseed rape (LEAR) (OECD 2011). The measured fatty acids in the dossier are reported in % based on the seed dry weight. No significant differences between ENTRY mean values over all sites (p-value ANOVA for ENTRY > 0.05) were found for most total fatty acids. The levels of erucic acid are very low in all samples (<0.01-0.03 %) and well below the 2% limit according to Codex alimentarius 2005 (Table 12 – appendix 2).

4.3 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 8-9 and Appendix 2, Table 1-1).

Mean values for the different agronomic data from the growth seasons 2001-2002 are summarised in tables 9, 12-15 – appendix 2. 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 8. Summary of parameters evaluated in the comparison of MS8 x 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	

Table 9. Summary of phenotypic and agronomic parameters evaluated in MS8 x 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			

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 x 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 x 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 x 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.

4.4 Conclusion

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 compositionally, agronomically and phenotypically equivalent to the conventional counterpart, except for the newly expressed barnase, barstar and PAT proteins.

In the Canadian field trials, however, compositional and phenotypic characteristics of oilseed rape MS8, RF3 and MS8 x RF3 were compared to null-segregant comparators. As negative segregants are derived from a GM organism, the VKM GMO Panel does not consider them appropriate conventional counterparts with a history of safe use. Data obtained from field trials with negative segregants are considered as supplementary information only.

Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of oilseed rape MS8 and RF3 to produce the hybrid MS8 x RF3 does not result in interactions that cause compositional, agronomic and phenotypic changes that would raise safety concerns.

5 Food and feed safety assessment

5.1 Product description and intended uses

The scope of notification C/BE/96/01) is for import and processing and feed uses of oilseed rape MS8 x RF3 and all derived products. In the human diet rapeseed is only used after processing into refined vegetable oil. The main by-product from oil processing, the mechanically and/or solvent extracted meal, is used as a protein rich feed for all classes of livestock (see chapter 3).

The genetic modification of oilseed rape MS8 x RF3 is intended to improve agronomic performance only and not intended to influence the nutritional properties, the processing characteristics and the overall use of oilseed rape as a crop.

5.2 Effects of processing

Western blot analyses of total protein extracts indicate that the PAT protein is detectable in all tissues, but the level of the protein was higher in green tissues, and only at trace levels in others. The Barnase and Barstar proteins are not detectable in rape seed. The PAT protein is detected in the seeds only at trace levels with a total amount of less than 0.001% of the total extractable protein. Further, PAT ELISA data indicate that degradation of the PAT protein occurs during the refining process (Van der Klis, 2002). The PAT protein is present at very low levels in the first fractions of processed seeds from MS8 x RF3 oilseed rape (the pressing cake and the extraction meal). PAT protein was detected only in trace amounts in toasted meal from oilseed rape and not detected in blended, degummed, refined, bleached and deodorized oil.

The applicant also provided data on the effect of temperature on recombinant PAT protein encoded by the *pat* gene. PAT-protein produced by *E. coli* was used to assess the stability of the protein following incubation for up to 60 minutes at 60˚, 75˚ and 90˚C. No degradation of the PAT protein was observed under these temperature conditions.

5.3 Toxicological assessment

Rapeseeds are only used in the human diet after processing into food grade vegetable oil. The presence of DNA in refined oils is generally very low, which is the only product intended for human consumption. The refining process for rapeseed oil also includes heating, solvent and alkali treatments that would be expected to remove and destroy DNA. The processing steps can also lead to the release of cellular enzymes (nucleases) that are responsible for degrading DNA into smaller fragments. The lack of intact DNA in the intended food products, oilseed rape oil reduces any risk of horizontal transfer of genetic material to cells in the human digestive tract as a result of the ingestion of these foods.The main side product from oil processing, the mechanical or solvent extracted meal, represents a source of protein in animal feeding.

The PAT-protein
Of the novel proteins expressed in oil seed rape MS8, RF3 and MS8 x RF3, only the PAT protein can be expected to be present in the food chain. The expression level of the PAT protein in the GM oilseed rape is low (5x10-4 % of total amount). *In vitro* digestion studies show rapid degradability of the PAT protein (EFSA 2005).

The total amino acid sequence of the PAT protein (De Beuckeleer, 2005) was compared to that of known toxins and allergens listed in 7 large public databases (SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept). The algorithm used for the homology comparison was BLASTP and the scoring matrix BLOSUM62. The criterion indicating potential toxicity or allergenicity was a 35 %

identity with a toxin or an allergen, on a window of 80 amino acids. The results of the epitope homology search showed no similarities between the PAT protein expressed by MS8 x RF3 and epitopes of known allergens based on a "100% identity over a linear contiguous 8 amino acid segment" matching criterion (Hérouet, 2005). Moreover, no potential glycosylation sites were identified in the PAT protein encoded by the *pat* gene. Based on these results, no evidence for any similarity to known toxic or allergenic proteins was found. As expected, the PAT protein presented only a high structural similarity with other non-toxic and non-allergenic PAT proteins (Hérouet, 2005). The overall homology search for the *pat* gene indicated significant homology only with other acetyltransferases.

Protein stability studies
The PAT protein encoded by the *pat* gene has an extremely short structural and functional stability under simulated gastric and intestinal conditions. PAT is not stable in an acidic environment. It is rapidly degraded (within 30 seconds) and inactivated in stomach fluids of cattle and pig. It is also rapidly and completely degraded in mammalian simulated gastric and intestinal fluids (between few and 30 seconds). These results confirm the safety of the PAT protein for human or animal consumption because the rapid degradation of the PAT protein greatly minimises the likelihood that this protein could survive in the digestive tract and be absorbed, thereby potentially eliciting a toxic or allergenic reaction.

5.3.1 Toxicological assessment of the newly expressed protein

5.3.1.1 Acute toxicity testing
Seed derived from MS8 x RF3 varieties is only different from counterpart seed by the presence of a novel protein, phosphinothricin acetyltransferase (PAT). Due to the low expression level of the PAT protein in MS8 x RF3 oilseed rape and the difficulties encountered in isolating a sufficient quantity of purified protein from GM oilseeds, protein safety studies were conducted with a PAT protein encoded by the pat gene (PAT/pat protein) and expressed in *E. coli*. Studies were undertaken demonstrating equivalence for the PAT protein (encoded by the *pat* gene) as it is expressed in MS8 x RF3 and the PAT protein (encoded by the pat gene) as it is produced by *E. coli.* Structural equivalence was demonstrated for PAT protein produced by *E. coli* and by MS8 x RF3 using SDS-PAGE and Western Blotting analysis. Both proteins showed indistinguishable electrophoretic mobility's and a molecular weight of approximately 22-24 kDa and can be considered equivalent. Functional equivalence between the two proteins is demonstrated by an enzymatic activity assay, showing identical substrate specificity. In addition, a glycosylation assay demonstrated that both proteins are not glycosylated, and N-terminal sequencing confirmed the identity of the proteins. Taken together, these results provide strong evidence that the protein produced in bacteria is indistinguishable from the same protein produced in plants (Hérouet et al. 2005b).

Acute intravenous exposure in rodents - PAT
Bayer Crop Sciences has performed an acute toxicity study of the PAT-protein in mice by a single intravenous administration. The study was performed in accordance with the principles of Good Laboratory of O.E.C.D. (Organization for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997, European Commission Directive 1999/1 I/EC, 1999, French decree n°98-1312, regarding Good Laboratory Practice, December 31, 1998, - E.P.A. (Environmental Protection Agency) • 40 CFR part 160 Federal Insecticide, Fungicide and Rodenticide Act (F1FRA): Good Laboratory Practice Standards: Final Rule, August 17, 1989, and Good Laboratory Practice Standards for Toxicology studies on Agricultural Chemicals, Ministry of Agriculture, Forestry and Fisheries (M.A.F.F.), notification 12 NohSan n°8628, (December 06, 2000).

The objective of this study was to assess the acute intravenous toxicity in OF1 mice of PAT (phosphoacetyl transferase) protein (>95% purity), a protein encoded by the *pat* gene. In addition, the acute intravenous toxicity of aprotinin (negative control) and melittin (positive control) were also compared. Groups of 5 female OF1 mice were administered either PAT protein, aprotinin or melittin

in physiological saline at dose levels of 1 and 10 mg/kg body weight. All animals were observed for clinical signs daily for fifteen days whilst their body weights were measured weekly. No clinical signs were noted in PAT protein-treated animals or in control groups throughout the study period. The body weight evolution was unaffected by the treatment with either PAT protein at 1 and 10 mg/kg or control substances up to Day 15.

At termination of the study period, animals were subjected to a necropsy including macroscopic examination. No treatment-related macroscopic abnormalities were detected in animals treated with either PAT protein at 1 and 10 mg/kg or control substances. There were no mortality or treatment-related toxic effects in female OF1 mice after acute intravenous administration of PAT (phosphoacetyl transferase) protein at 1 and 10 mg/kg.

The positive control (melittin), at 10 mg/kg, induced 100% mortality. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 and 10 mg/kg showed no visible signs of systemic toxicity.

There is no acute toxicity from the PAT protein, and the molecular characterization of MS8 x RF3 oilseed rape did not reveal unexpected consequences of the genetic modification, e.g. ORF sequences coding for potential toxic proteins (EFSA/GMO/RX/MS8/RF3). Additional results from new bioinformatic studies, comparing amino acid sequences in up-to-date databases confirm that no homologies exist between the newly expressed proteins and known toxic proteins or and allergens, therefore, do not indicate safety concerns with regard to potential production of new toxins or allergens (EFSA/GMO/BE/2010/81). A battery of tests designed to evaluate the PAT protein for characteristics associated with food allergens and toxins raised no concern (Hérouet, 2005). The PAT protein shares no sequence homology with known allergens and toxins and is not stable in digestive environments.

5.3.1.2 Sub-chronic oral toxicity testing

Repeated dose 14-day oral toxicity study in rodents.
Bayer Crop Sciences has performed a sub-chronic oral toxicity study of the PAT-protein in rats. The study was performed in accordance with the principles of Good Laboratory of O.E.C.D. (Organization for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1992. Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 and the Japanese Ministry of Agriculture, Forestry and Fisheries: On Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals, Agricultural Production Bureau, 59 NohSan Notification Number 3850, August 10, 1984.

Test guidelines: The study procedures mostly conform to OECD Guidelines for Testing of Chemicals, number 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents", adopted by the Council on July 27, 1995. According to the OECD guidelines the duration of exposure should normally be 28 days although a 14-day study may be appropriate in certain circumstances; justification for use of a 14-day exposure period should be provided. The duration of this repeated dose oral toxicity was 14-day exposure period. No justification for using 14-days has been found in the dossier of the applicant.

Wistar rats of group 1 received a standard rat diet (Kliba 343) and rats of groups 2, 3 and 4 a standard rat diet with low protein, which was adjusted to a protein content similar to that of group 1 by using soya bean derived protein (soyamin) at concentrations of 45'000 ppm for group 2, 0 ppm for group 3 and 50'000 ppm for group 4. PAT-protein was administered by feed admixture in powdered standard low protein diet to Wistar rats at concentrations of 0 (group 1), 5000 (group 2), 50000 (group 3) and 0 ppm (group 4) for a period of 14 days. The study comprised four groups of each five male and five female rats. The average intake of PAT-protein over the entire treatment were: males: 0, 712 and 7619 mg/kg/day; females: 0, 703 and 7965 mg/kg/day, see table 10.

Table 10. The nominal dietary concentrations of PAT-PROTEIN and SOYAMIN (soya bean derived protein) and the mean intake of PAT-PROTEIN calculated over the entire treatment period.

Group	Protein content in diet			Average Intake of PAT-Protein	
	Type of diet	Soyamin (ppm)	PAT-Protein (ppm)	Males (mg/kg/day)	Females (mg/kg/day)
1	standard	0	0	0	0
2	low protein	45'000	5'000	712	708
3	low protein	0	50'000	7619	7963
4	low protein	50'000	0	0	0

The results show no unscheduled deaths, no clinical signs were noted and food consumption and body weights were unaffected by treatment. No treatment-related changes were seen in hematology or urinalysis parameters. Organ weight data, macroscopical and microscopical findings did not distinguish treated groups from controls.

The only changes which might be attributed to treatment were observed in clinical biochemistry parameters. They consisted of a slightly lower glucose level in males of group 4, slightly higher total cholesterol and phospholipid levels in male rats of groups 2, 3 and 4 and slightly higher triglyceride level in females of group 4 when compared with rats of group 1. Animals of group 4 received no PAT-protein but - with respect to the protein content - a diet most similar to that of groups 2 and 3. The above changes are according to the applicant considered to reflect differences in the dietary composition and to be unrelated to PAT Protein itself.

Comparing the increased total cholesterol and phospholipid levels between group 3 (low protein diet + 50000 ppm PAT-protein) and group 4 (low protein diet + 50000 ppm soya protein) they are found to be in a similar range. This may suggest a similar nutritional value of both proteins. Based on the results of this study, there is no evidence of toxicity for PAT-protein when administered to rats in their feed at dietary concentrations up to 50000 ppm for a period of 14 days.

5.3.2 Toxicological assessment of the whole GM food/feed

5.3.2.1 Feeding study on male broiler chickens

A 42-day feeding study was carried out on broiler chickens (420 Ross chickens). Birds were divided in three groups (140 chickens per group). The study was conducted according to the Springborn Smithers protocol entitled "Broiler Chicken Feeding Study with Ms8/Rf3 Rapeseed" Springborn Smithers Protocol No.: 102003/OECD/JMAFF/Broiler Chicken. The methods described in this protocol comply with the U. S. Environmental Protection Agency's (EPA) Good Laboratory Practice Standards as set forth under the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR, Part 160, 1989), OECD Principles of Good Laboratory Practice (OECD, 1997) and JMAFF (notification 11 Nousan N 6283 (October 01, 1999) modified by notification 12 Nousan N 8628 (December 06, 2000) with the exceptions that are mentioned in the "Good Laboratory Practice Compliance Statement".
Analyses for compositional content and presence of phytic acid, heavy metals, mycotoxins, bacteria, and pesticides were conducted by Woodson-Tenent Laboratories. Crop-specific anti-nutrients (erucic acid, glucosinolates) were analysed by Bayer Crop Science.

Birds were housed 10 per pen (replicate), 14 replicates per group, and 140 birds per group. According to the supplier the birds were assigned to cages for which gender assignment had been determined

based on the gender designation specified by the supplier, such that there were seven cages of each gender per group. However, internal exams made during post-mortems and after study termination (for all surviving chickens) revealed a discrepancy in the hatch-day gender determination specified by the supplier (Group A: 19F, 23M; Group B: 14F, 28M; Group C: 19F, 23M). Therefore, most cages contained animals of both genders, with varying gender ratios among the cages.

Effects on health, survival, weight gain, feed consumption, feed conversion, marketable carcass and muscle (breast, thigh, leg, and wing) weights and percent yields were evaluated. Birds were fed diets containing 10 % by weight MS8 x RF3 oilseed rape treated with the target herbicides, 10 % by weight MS8 x RF3 oilseed rape untreated, or fed 10 % by weight of a commercial non-GM oilseed rape variety with background genetics similar to MS8 x RF3. At the end of the study, body weight and feed intake were determined, the birds slaughtered, and carcass parameters determined. Average feed consumption per bird per week during week 1 ranged from 105.7 g (Group C) to 140.1 g (Group C). Average feed consumption measured at the end of week 6, and study termination had reached ranges of 804.7 g (Group B) to 1260.6 g (Group A). Average total feed consumption over the entire 42-day study ranged from 3430.3 g per bird (cage 30, Group A) to 4101.8 g per bird (cage 28, Group C). The average feed to body weight conversion ratio was 1.8 for all groups. Feed consumption and feed conversion were within the normal range. The statistical analyses validate that there is no change between groups.

Statistical analyses were conducted separately on two data sets. The first included those dependent variables for which the cage average was the test replicate.These variables included 1) feed consumption, and 2) feed to body weight conversion. A third variable, weight gain, was initially intended to be included in this category (cage as the replicate). However, since the cages contained mixed genders, and since the gender of all birds was determined by internal examination at the end of the study, this variable was included in the second data set.

According to the applicant there were no significant differences in weight gain, feed consumption, feed conversion or carcass, breast, thigh, leg and wing weight among treatment groups. Based on daily observations, it appeared that males were more aggressive around the feeder, and the larger males appeared most dominant. In group A, where more males died during the study (primarily from heart failure) the remaining males gained the most weight. These larger males were more dominant around the feeders, allowing the smaller females less time to feed. These females gain less weight than females in Groups B and C. The effect of this phenomenon was reflected in the results of the tests for Group x Gender interactions in the ANOVA for wing weight.

The applicant working hypothesis was that the ANOVA for feed consumption among groups (cages with mixed gender) did not detect a difference in the mean values among groups because the larger males in Group A cages consumed more feed, offsetting the reduced feed consumption by the smaller females in the same cages, leaving the group means essentially equal. According to the applicant this result was deemed unrelated to the characteristics of the three feed types.

Accordingly the broiler feeding study supports the results of the comparative compositional analysis, and that this indicates that oilseed rape MS8 × RF3 is as nutritious as a commercial non-GM oilseed rape with a genetic background similar to oilseed rape MS8 × RF3.
VKM comments: No histopathology or other clinical parameters are investigated in this feeding study. Therefore this study provides only limited evidence for safety.

5.3.2.2 Rabbit digestibility study

Thirty, seven-week old rabbits were used to determine the nutritive value of two oilseed rapes: a non-transgenic control and the MS8 x RF3 transgenic oilseed rape. The experiments were carried out according to “European reference method for *in vivo* determination of diet digestibility in rabbits”. The inclusion level, at the expense of all basal ingredients, amounted 30%. The control and the

transgenic oilseed rape diet showed no significant differences in protein content. However, the fat content was 3% lower in the non-transgenic oilseed rape. A preliminary adaption period of 10 days was carried out before the 4 day balance trial. Especially during the first 3 days of the adaption period, rabbits fed oilseed rape diets showed a severely decreased feed intake (45% of basal diet) and consequently lower weight gains. After one week the intake reached a normal level taking into accouint the increased dietary content. No significant difference in intake pattern between oilseed rapes was found (Maertens et al. 1996).

5.4 Allergenicity assessment

5.4.1 Assessment of allergenicity of the newly expressed protein

It is an accepted approach for a safety assessment to compare the characteristics of a novel protein with a number of parameters that are common to food allergens. A search of the current amino acid sequence databases for homology with known allergens provides another point of review. Since most allergens may resist gastric acidity and digestive proteases, and may remain stable in food processing (heating). However, as mentioned earlier the PAT protein is rapidly and completely degraded in mammalian simulated gastric and intestinal fluids (between few and 30 seconds).

An epitope sequence homology search of the PAT protein, subdivided into 8 amino acid blocks, to known epitopes belonging to known allergens has been performed. The BLASTP algorithm and the BLOSUM62 scoring matrix were also used for this search. The criterion indicating potential allergenicity was a match of at least eight contiguous identical amino acids with a known allergenic epitope. No sequence similarities with an allergenic epitope were observed (Hérouet 2002). Further, an in silico approach enabled the search of the potential N-glycosylation sites often found on allergens. The results showed that such sites of potential post-translational glycosylations were not found on the PAT protein (Hérouet, 2002). In general, IgE binding epitopes are known to be commonly robust to treatment with heat and electroblotting on nitrocellulose. For this reason, the stability of food allergens to heat processing argues for importance of linear, continuous epitopes in assessing potential allergenicity. When treated at temperatures up to 90°C for 60 minutes, the PAT protein (encoded by pat gene) remains detectable by SDS-PAGE (Esdaile 2002c). This shows the importance of the epitope homology search, which found no similarities with known allergenic epitopes. Thus, there is a reasonable certainty of no allergenicity concern associated with the presence of PAT protein.

The PAT protein is the only newly expressed protein present in oilseed rape MS8, RF3 and MS8 x RF3 seed and pollen. Barnase and barstar proteins are expressed only in the tapetum cells of the flower buds and therefore will not occur in food or feed derived from oilseed rape MS8, RF3 and MS8 x RF3 seed or pollen. The *in vitro* digestibility studies showed that the PAT protein was rapidly degraded.

Bioinformatic studies previously evaluated revealed no relevant similarity between the newly expressed proteins PAT, barnase and barstar and known IgE-allergens (EFSA-GMO-RX-MS8-RF3 (2008), EFSA 2009a). The study was carried out comparing the PAT protein, subdivided into 8 amino acid blocks, with potential epitopes of all probable allergens present in the Allergen database. The *in silico* approach enabled the search of potential N-glycosylation sites present in the PAT protein. The results showed that such sites of potential post-translational glycosylations were not found in the PAT protein. These finding reinforces the fact that the PAT protein does not have glycosylation sites, a characteristic associated with known food allergenic proteins. Additional results from new bioinformatics studies, comparing amino acid sequences in up-to-date databases confirm no homologies exist between the newly expressed proteins and known toxic proteins or allergens and, therefore, do not indicate any safety concerns with regard to potential production of toxins or allergens, and thus confirm the results of the previous study.

Additionally, the allergenicity of the whole GM plant can theoretically be increased by unintended changes at the insertion sites by modifying the expression of endogenous genes (potential allergens) or by producing new allergens. However, bioinformatics analyses of the DNA sequence at the insertion sites did not indicate: (i) changes in the expression of endogenous genes; or (ii) creation of open reading frames at the insert–plant DNA junctions that are likely to be translated into allergenic peptides. Considering all information available, there is no evidence that the genetic modification might alter the pattern of expression of endogenous proteins (potential IgE-allergens) in the oilseed rape MS8, RF3 and MS8 x RF3 and, thereby, significantly change the overall allergenicity of the whole GM plant.

5.4.2 Assessment of the allergenicity of the whole GM plant

Oilseed rape (*Brassica napus* L.) is not considered an allergenic food. However, oilseed rape contains storage proteins that share amino sequence similarity to mustard allergens. Plants are known to naturally produce toxins and allergens which often serve the plant as natural defense compounds against pests and pathogens. In the past the inclusion of oilseed rape products in human food or animal feed was limited due to the presence of some antinutrients that could act as toxic compounds. These antinutritional and toxic factors are glucosinolates, erucic acid and phytic acid. Erucic acid is present in the oil and glucosinolates are present in the meal. Breeding efforts have reduced the levels of both erucic acid and glucosinolates resulting in "double zero" varieties (Europe) and "canola"-type varieties (Canada). In Europe, "double zero" rapeseed varieties are defined as those producing seed with a maximum glucosinolate content of 25 μmoles/g (seed weight) and with a moisture content of 9% and, having erucic acid content of not more than 2% of the total fatty acid content. Canola is defined as having less than 2% erucic acid in the oil and less than 30 μmol/g glucosinolates in the air-dried, oil-free meal. AC Excel, the recipient variety of T45, meets these criteria. The transformation process did not result in levels significantly different from the recipient variety.

Rapeseed oil and meal are currently considered not to contain common food toxins or antinutritional components of concern for human and animal health, because either the product only has minor amounts of these active compounds or their levels decrease (or they even disappear) during processing. A consideration of specific food safety issues did not identify food allergenic potential as one outcome that would cause concern for human consumption. Edible oils that are refined, bleached and deodorized do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. Therefore, no allergic reaction is expected from its current use pattern.

5.4.3 Assessment of the allergenicity of proteins from the GM plant

Oilseed rape is related to *B. juncea* and *B. nigra* from which brown and black mustard respectively are obtained. Mustard is one of twelve known food allergens that must be labelled when used in food production under EU legislation (Matportalen 2013). Cross-reactive ELISA-studies have been used for detection mustard in foods (Aider & Barbana 2011). A rapeseed sample containing rapeseed proteins displayed strong reactivity in the mustard Elisa. The results show that it was a 2 S storage albumins in oilseed rape which reacted in the mustard ELISA. This was expected since 2 S albumin in oilseed rape share 94 % sequence similarity with 2 S mustard albumins. The 2 S albumin exhibited structural relationship with napin-like 2 S proteins from oilseed rape seeds. IgE and IgG cross-reactivity between oilseed rape seed and mustard allergens was demonstrated (Aider & Barbana, 2011).

Since 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, potential risk of allergic reactions to these oilseed rape proteins should have been performed by the applicant.
A consideration of specific food safety issues did not identify food allergenic potential as one outcome that would cause concern for human consumption. Edible oils that are refined, bleached and

deodorized do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. The PAT-protein was not detected in blended, degummed, refined, bleached and deodorized oil, and therefore no allergic reaction is expected from the use of rapeseed oil.

Isolated rapeseed proteins.
In 2010 the company Archer Daniels Midland/Burcon NutraScience Corporation notified the FDA of the GRAS self-affirmation of the cruciferin-rich canola/rapeseed protein isolate (Puratein (R)) and the napin-rich canola/rapeseed protein isolate (Supertein™) extracted from canola/rapeseed species, low in glucosinolates and erucic acid. These protein isolates have been classified as GRAS substances by FDA.

In 2011 the company BioExx Speciality Proteins, Ltd. notified the FDA of the GRAS self-affirmation of two canola protein products - canola protein isolate (IsolexxTM) and hydolyzed canola protein isolate (Vitalexx™). These protein isolates have also been classified as GRAS substances by FDA.

These protein isolates contain proteins which share considerable sequence homology to known allergens from yellow mustard (*Sinapis alba*) and Indian mustard seeds.

A screnning test with skin prick test (SPT) of Finnish children showed that 28 out of 1887 young children had positive SPT to oilseed rape and turnip rape (Poikonen et al 2006). In another study by the same group it was shown that 2S albumins, or napins, in oilseed rape are potential allergens (Puumalainen et al 2006).

The GMO Panel finds that there is ucertainty whether individuals who are allergic to mustard proteins will also react upon consumption of products containing these rapeseed/canola proteins.

5.4.4 Adjuvanticity

Adjuvants are substances that, when co-administered with an antigen increases the immune response to the antigen and, therefore, might increase the allergic response as well (EFSA 2011). In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity (EFSA 2010b).

5.5 Nutritional assessment of GM food/feed

Low erucic acid rapeseed seeds are processed into two major products: oil and meal. The oil and meal are further manufactured into a wide variety of products for human-, agricultural-, as well as industrial use (OECD 2011).

Human food use of whole seeds and flour of low erucic acid rapeseed have been reported anecdotally, and a sensory evaluation of canola greens has been published (Miller-Cebert et al. 2009). Food use of protein fractions from low erucic acid rapeseed meal has in the past received little attention for human nutrition due to their high level of antinutrients (Tan et al 2011). However, newer technologies can eliminate such compounds (Fleddermann et al 2012).

The meal left after extraction of oil from the seed is used as a high (36-44%) protein feed source for all classes of livestock, poultry and fish (OECD 2011). Because low erucic acid rapeseed meal contains 30% hulls, it has a high fibre content, which limits its use to approximately 15% of the total diet in monogastric diets. Higher inclusion rates are practical in ruminant rations, especially for dairy cows. Low erucic acid rapeseed meal can be used as the sole protein supplement for ruminants. De-hulled

low erucic acid rapeseed meal has the potential to compete with soybean meal in swine and poultry diets (OECD 2011).

5.6 Conclusion

Whole food feeding studies in broilers have not indicated any adverse health effects of oilseed rape MS8 x RF3. These studies also indicate that oilseed rape MS8 x RF3 is nutritionally equivalent to conventional oilseed rape. The PAT protein do not show sequence resemblance to other known toxins or IgE allergens, nor has PAT been reported to cause IgE mediated allergic reactions.

Based on the current knowledge, the VKM GMO Panel concludes that oilseed rape MS8 x RF3 is nutritionally equivalent to conventional oilseed rape varieties, and that it is unlikely that newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from oilseed rape MS8 x RF3 compared to conventional oilseed rape.

6 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 x RF3.

6.1 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 2012).

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 2012). The relative importance of wind versus insect pollination is unclear and probably varies with location and weather. The rape pollen seeds have features that are typical of insect pollination being relatively large (32-33 µm), heavy and sticky (OECD 2012; 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).

6.2 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 2012), 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. 2008).

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 lants/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.

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

6.3 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.

6.3.1 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.

In conclusion, the VKM GMO Panel considers it unlikely that the introduced genes in oilseed rape MS8, RF3 and MS8 x RF3 will horizontally transfer and integrate with the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible event of transfer of the *barnase*, *barstar* as well as *bar* genes from MS8, RF3 and MS8 x RF3 to soil bacteria, no novel property would be introduced into, nor expressed by the soil microbial communities as sequence-similar genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

6.3.2 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.

6.3.2.1 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.

6.3.2.2 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 2012). 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 2012).

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 24 (OECD 2012). A summary of some of these studies are presented in the following paragraphs and discussed in more details in the Appendix 3.

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 2012).

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 2012).

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.

6.4 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.

6.5 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.

6.6 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.

6.7 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.

6.8 Conclusion

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.

7 Data gaps

- Routes of import, transport and processing of oilseed rape seeds in Norwegian environments, and quantitative considerations of the potential of spillage.
- Established whether feral populations of oilseed rape are short-lived or have a more permanent nature. Since the places where most substantial losses occur are most likely to show the first initial populations, particularly these places should be identified and studied.
- The 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.
- Lack of 90 day study on rats.

8 Conclusions

Molecular characterisation

The oilseed rape hybrid MS8 x RF3 is produced by conventional crossing. The parental lines MS8 and RF3 are described in the documentation provided by the applicant, and a number of published studies support their data. The data submitted by the applicant justify the conclusion 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 x 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 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 compositionally, agronomically and phenotypically equivalent to the conventional counterpart, except for the newly expressed barnase, barstar and PAT proteins.

In the Canadian field trials, however, compositional and phenotypic characteristics of oilseed rape MS8, RF3 and MS8 x RF3 were compared to null-segregant comparators. As negative segregants are derived from a GM organism, the VKM GMO Panel does not consider them appropriate conventional counterparts with a history of safe use. Data obtained from field trials with negative segregants are considered as supplementary information only.

Based on the assessment of available data, the VKM GMO Panel is of the opinion that conventional crossing of oilseed rape MS8 and RF3 to produce the hybrid MS8 x RF3 does not result in interactions that cause compositional, agronomic and phenotypic changes that would raise safety concerns.

Food and feed risk assessment

Whole food feeding studies in broilers have not indicated any adverse health effects of oilseed rape MS8 x RF3. These studies also indicate that oilseed rape MS8 x RF3 is nutritionally equivalent to conventional oilseed rape. The PAT protein do not show sequence resemblance to other known toxins or IgE allergens, nor has PAT been reported to cause IgE mediated allergic reactions.

Based on the current knowledge, the VKM GMO Panel concludes that oilseed rape MS8 x RF3 is nutritionally equivalent to conventional oilseed rape varieties, and that it is unlikely that newly expressed proteins introduce a toxic or allergenic potential in food and feed derived from oilseed rape MS8 x RF3 compared to conventional oilseed rape.

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

Based on current knowledge, the VKM GMO Panel has not identified toxic, allergenic or altered nutritional properties of oilseed rape MS8, RF3 and MS8 x RF3 or its processed products compared to conventional oilseed rape.

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

References

Abrahamsen U (2009) Sortsforsøk I vårraps. I: Jord og plantekultur. Bioforsk FOKUS 4 (1):152-154

Abrahamsen U (2011) Sortsforsøk I vårraps. I: Jord og plantekultur. Bioforsk FOKUS 6 (1):128-130

Aider M, Barbana C (2011) Canola proteins: composition, extraction, functional properties, bioactivity, application as food ingredient and allergenicity - a practical and critical review. Trends Food Sci Tech. 22: 21–39

Beckie HJ, Warwick SI, Nair H, Séguin-Swartz G (2003) Gene flow in commercial fields of herbicide-resistant canola. Ecological Applications 13: 1276-1294

Beckie HJ, Warwick SI (2010) Persistence of an oilseed rape transgene in the environment. Crop Protection 29: 509-512

Bensasson D, Boore JL, Nielsen KM (2004) Genes without frontiers. Heredity 92: 483-489

Bing DJ, Downey RK, Rakow GFW (1991) Potential of gene transfer among oilseed *Brassica* and their weedy relatives. In: DJ McGregor (ed) Proc 8th Int. Rapeseed Congr. Saskatoon. Canada. pp. 1022-1027

Bing DJ, Downey RK, Rakow GFW (1995) An evaluation of the potenstial of intergeneric gene transfer between *Brassica napus* and *Sinapis arvensis*. Plant Breeding 114: 481-484

Bing DJ, Downey RK, Rakow GFW (1996) Hybridizations among *Brassica napus. B. rapa* and *B. juncea* and their two weedy relatives *B. nigra* and *Sinapis arvensis* under open pollination conditions in the field. Plant Breeding 115: 470-473

CERA (2013) Center for Environmental Risk Assessment. GM Database for safety information. http://cera-gmc.org/index.php?action=gm_crop_database

Chévre AM, Eber F, Baranger A, Kerlan MC, Barret P, Festoc G, Vallée P, Renard M (1996) Interspecific Gene Flow as a Component of Risk Assessment for Transgenic Brassicas. Acta Hort. 407: 169-179

Claessen D, Gilligan CA, Lutman PJW, van den Bosch F (2005a) Which traits promote persistence of feral GM crops? Part 1: implications of environmental stochasticity. Oikos 110: 20-29

Claessen D, Gilligan CA, van den Bosch F (2005b) Which traits promote persistence of feral GM crops? Part II: implications of metapopulation structure. Oikos 110: 30-42

Crawley MJ, Brown SL (1995) Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. Proceedings of the Royal Society B – Biological Sciences, 259: 49–54

Crawley MJ, Brown SL, Hails RS, Kohn DD, Rees M (2001) Transgenic crops in natural habitats. Nature 409: 682-683

Crawley MJ, Brown SL (2004) Spatially structured population dynamics in feral oilseed rape. Proceedings of the Royal Society B – Biological Sciences 271: 1909–1916

Crawley MJ, Hails RS, Rees M, Kohn DD, Buxton J (1993) Ecology of transgenic oilseed rape in natural habitats. Nature 363: 620-623

Daniels R, Boffey C, Mogg R, Bond J, Clarke R (2005) The potential for dispersal of herbicide tolerance genes from genetically-modified. herbicide-tolerant oilseed rape crops to wild relatives. Final report to DEFRA (2005). Dorset. 23s.

Darmency H, Fleury A (2000) Mating system in *Hirschfeldia incana* and hybridisation to oilseed rape. Weed Research 40: 231-238

D'Hertefelt T, Jørgensen RB, Petterson L (2008) Long term persistence of GM oilseed rape in the soil seed bank. Biology Letter 4:314-317

de Vries J, Wackernagel W (2002) Integration of foreign DNA during natural transformation of *Acinetobacter* sp. by homology-facilitated illegitimate recombination. The Proceedings of the National Academy of Sciences USA 99: 2094-2099

De Beuckeleer M (2005) Description of the amino acid sequence of the PAT protein encoded from the *bar* gene. Bayer BioScience NV.5 s.

Devos Y, Reheul D, De Schriver A, Cors F. Moens W (2004) Management of herbicide-tolerant oilseed rape in Europe: a case study on minimizing vertical gene flow. Environmental Biosafety Research 3: 135-148.

Devos Y, Schriver AD, Reheul D (2009) Quentifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives. Environ Monit Assess 149: 303-322

Devos Y, Hails RS, Messéan A, Perry JN, Squire GR (2012) Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? Transgenic Res 21:1-21

Eastham K, Sweet J (2002) Genetically modified organisms (GMO): The significance of gene flow through pollen transfer. Environmental issue report. No 28. European Environment Agency (EEA). Copenhagen. http://reports.eea.eu.int/environmental_issue_report_2002_28/en.

EC (1999) Commission Regulation (EC) 2316/1999 of 22 October 1999 laying down detailed rules for the application of Council Regulation (EC) No 1251/1999 establishing a support system for producers of certain arable crops. OJ, L 280, 43-56.

EFSA (2004) Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal. 48: 1-18 http://www.efsa.europa.eu/en/science/gmo/gmo_opinions/384.html.

EFSA (2005) Opinion of the Scientific Panel on genetically modifies organisms on a request from the Commission related to the application (Reference C/BE/96/01) for the placing on the market of glufosinate-tolerant hybrid oilseed rape MS8 x RF3, derived from genetically modified parental lines (MS8. RF3). for import and processing for feed and industrial uses, under Part C of Directive 2001/18/EC from Bayer CropScience. The EFSA Journal 281: 1-23

EFSA (2009a) Scientific opinion on application (EFSA/GMO/RX/MS8/RF3) for renewal of the authorisation for continued marketing of existing (1) food and food ingredients produced from genetically modified oilseed rape MS8, RF3 and MS8 x RF3, and (2) feed materials produced from genetically modified glufosinate-tolerant oilseed rape MS8, RF3 and MS8 x RF3, under Regulation (EC) No 1829/2003 from Bayer. The EFSA Journal 7(9): 1318

EFSA (2009b) Use of antibiotic resistance genes as marker genes in genetically modified plants. Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ). The EFSA Journal 1034: 1-82 http://www.efsa.europa.eu/cs/BlobServer/Statement/gmo_biohaz_st_ej1108_ConsolidatedARG_en.pdf?ssbinary=true

EFSA (2010a) Guidance on the environmental risk assessment of genetically modified plants. Scientific option from the EFSA Panel on Genetically Modified Organisms (GMO). The EFSA Journal 8 (11):1-111 http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf

EFSA (2010b) Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Panel on Genetically Modified Organisms (GMO Panel). EFSA Journal 8 /7): 1700 http://www.efsa.europa.eu/en/efsajournal/doc/1700.pdf

EFSA (2011a) Guidance for risk assessment of food and feed from genetically modified plants. The EFSA Journal. 9(5): 2150. http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf

EFSA (2011b) EFSA Panel on Genetically Modified Organisms (GMO). Scientific Opinion on Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. EFSA Journal 9(5):2149

EFSA (2011c) Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316

EFSA (2012) Scientific opinion on application (EFSA/GMO/BE/2010/81) for the placing on the market of genetically modified herbicide-tolerant oilseed rape MS8. RF3 and MS8 x RF3 for 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) under Regulation (EC) No 1829/2003 from Bayer. The EFSA Journal 10(9): 2875

Elling B, Neuffer B, Bleker W (2009) Sources of genetic diversity in feral oilseed rape (*Brassica napus*) populations. Basic and Applied Ecology 10: 544-553

EU-COM (2013) EU Oilseeds overview 2009/2010 to 2013/2014. European Commission. Directorate General for Agriculture and Rural Development. Unit C5.

Esdaile DG (2002) Phosphinotricin Acetyltransferase (PAT) pat Gene Product, Heat Stability Study. Bayer CropScience. Internal Report. 31 pages. M-131221-03-1

FAOSTAT (2013) FAOSTAT – Global harvested area of rapeseed in 2011. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Fledderman M, Fechner A, Rößler A, Bähr M et al (2013) Nutritional evaluation of rapeseed protein compared to soy protein for quality, plasma amino acids, and nitrogen balance e A randomized cross-over intervention study in humans. Clin Nutr 32(4): 519-526

Frello S, Hansen KR, Jensen J, Jørgensen RB (1995) Inheritance of rapeseed (*Brassica napus*) specific RAPD markers and a transgene in the cross *B. juncea* x (*B.juncea* x *B. napus*). Theoretical Applied Genetics 91: 193-200

Gain Report (2011) EU 27 Rapeseed production somewhat better than expected. GAIN Report Global Agricultural Information Network. USDA Foreign Agricultural Service

Gain Report (2012) EU 27 Oilseeds and Products Annual. Despite winter Kill, Modest Rebound in EU-27 Rapeseed Productiontion. Gain report number: E70016.

Garnier A, Deville A, Lecomte J (2006) Stochastic modelling of feral plant populations with seed immigration and road verge management. Ecological Modelling 197: 373–382

Gruber S, Pekrun C, Claupein W (2004) Population dynamics of volunteer oilseed rape (*Brassica napus* L.) affected by tillage. European Journal of Agronomy 20: 351–361

Gruber S, Bühler A, Möhring J, Claupein W (2010) Sleepers in the soil-vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. Eur J Agron 33: 81-88

Guéritaine G et al. (2002) Fitness of Backcross Six of Hybrids between Transgenic Oilseed Rape (*Brassica napus*) and Wild Radish (*Raphanus raphanistrum*). Molecular Ecology 11: 1419-1426

Gulden RH, Shirtliffe SJ, Thomas AG (2003) Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. Weed Science 51: 83-86

Gulden RH, Thomas AG, Shirtliffe SJ (2004a) Relative Contribution of Genotype, Seed Size and Environment to Secondary Seed Dormancy Potential in Canadian Spring Oilseed Rape (*Brassica napus*). Weed Res. 44: 97-106

Hails RS, Rees M, Kohn DD, Crawley MJ (1997) Burial and seed survival in *Brassica napus* subsp. *oleifera* and *Sinapis arvensis* including a comparisation of transgenic and non-transgenic lines of the crop. Proceedings of the Royal Society B 264: 1-7

Halfill MD, Zhu B, Warwick SI, Raymer PL, Millwood RJ, Weissinger AK, Stewart CN (2004) Hybridization and backcrossing between transgenic oilseed rape and two related weed species under field conditions. Environmental Biosafety Research 3: 73-81

Hall LM, Rahman MH, Gulden RH, Thomas AG (2005) Volunteer oilseed rape: will herbicide-resistance traits assist ferality? In Crop Ferality and Volunteerism (Gressel J ed.). pp 59-79. Boca-Raton. FL: CRC Press.

Hansen LB, Siegismund HR, Jørgensen RB (2001) Introgression between oilseed rape (*Brassica napus* L.) and its weedy relative *B. rapa* L. in a natural population. Gen Res Crop Evol 48:621-627

Hérouet C (2002a) Assessment of the toxicity and allergenicity of the *bar* gene and its product the PAT protein. Study Number SA02218. Internal Bayer CropScience Report. September 18, 2002. 44 Pages.

Hérouet C (2002b) *Phosphinothricin-Acetyl-Transferase* (*PAT*)- *Pat* gene product. Overall amino acid sequence homology search with known toxins and allergens. Study Number SA02254. Internal Bayer CropScience Report. July 12, 2002. 85 Pages.

Hérouet C, Esdaile DJ, Mallyon BA, Debruyne E, Schulz A, Currier T, Hendrickx K, van der Klis R-J, Rouan D (2005) Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the pat and bar sequences that confer tolerance to glufosinate-ammonium herbicide in transgenic plants. Regul Toxicol Pharmacol 41; 134–149.

Hoel B, Abrahamsen U, Strand E, Åssveen M, Stabbetorp H (2013) Tiltak for å forbedre avlingsutviklingen i norsk kornproduksjon. Bioforsk Rapport 8(14). 95 s.

Jørgensen RB (1999) Gene flow from oilseed rape (*Brassica napus*) to related species. British Crop Protection Council. Farnham. Surrey. UK. Pp 117-124

Jørgensen RB, Andersen B (1994) Spontanous hybridization between oilseed rape (*Brassica napus*) and weedy *B. campestris* (*Brassicaceae*): a risk of growing genetically modified oilseed rape. Am J Bot 81: 1620-1626

Jørgensen RB, Andersen B, Landbo L, Mikkelsen TR (1996) Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy relatives. Act Hort 407: 193-200

Jørgensen RB, Andersen B, Hauser TB, Landbo L, Mikkelsen TR, Østergård H (1998) Introgression of crop genes from oilseed rape (*Brassica napus*) to related wild species-an avenue for the escape of engineered genes. Acta Hort 459: 211-217

Jørgensen T, Hauser TP, Jørgensen RB (2007) Adventitious presence of other varieties in oilseed rape (*Brassica napus*) from seed banks and certified seed. Seed Science Research 17: 115-125

Jørgensen RB, Hauser T, D'Hertefeldt T, Andersen NS. Hooftman D (2009) The variability of processes involved in transgene dispersal–case studies from *Brassica* and related genera. Environ Sci Pollut Res 16:389–395

Kawata M, Murakami K, Ishikawa T (2009) Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. Published online: 3 December 2008. Environ Sci Pollut Res 16:120–126

Knispel AL, McLachlan SM, Van Acker RC, Friesen LF (2008) Gene Flow and Multiple Herbicide Resistance in Escaped Canola Populations. Weed Science. 56(1):72-80. http://www.bioone.org/doi/full/10.1614/WS-07-097.1

Knispel AL, McLachlan SM (2010) Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba. Canada. Environ Sci Pollut R 17: 13-25

Landbo L, Andersen B, Jørgensen RB (1996) Natural hybridization between oilseed rape and a wild relative: hybrids among seeds from weedy *B. campestris*. Hereditas 125: 89-91

Lefol E, Danielou V, Darmency H (1996) Gene dispersal from transgenic crops: II. Hybridization between oilseed rape and the wild hoary mustard. Sexual Plant Reprod 9: 189-196

Lefol E, Séguin-Swartz G, Downey RK (1997) Sexual hybridisation in crosses of cultivated *Brassica* species with the crusifers *Erucastrum gallcum* and *Raphanus raphanistrum*: potential for gene introgression. Euphytica 95: 127-139

Lecomte J, Bakker Jørgensen R, Bartkowiak-Broda I, Devaux C, Dietz-Pfeilstetter A, Gruber S et al. (2007) Gene flow in oilseed rape: what do the datasets of the SIGMEA EU Project tell us for coexistence? In: Stein A. Rodriguez-Cerezo E (eds) Books of abstracts of the third International Conference on Coexistence between Genetically Modified (GM) and non-GM-based Agricultural Supply Chains. European Commission. pp 49-52

Lid J, Lid DT (2005) Norsk flora. Det Norske Samlaget. Oslo. 7. utgave. ISBN: 82-521-6029-8. 1230s.

Liu YB, Wei W, Ma KP, Darmency H (2010) Backcrosses to *Brassica napus* og hybrids between *B. juncea* and *B. napus* as a source of herbicide-resistant volunteer-like feral populations. Plant Science 179: 459-465

Londo JP, Bautista NS, Sagers CL, Lee EH, Watrud LS (2010) Glyphosate drift promotes changes in fitness and transgene gene flow in canola (*Brassica napus*) and hybrids. Ann Bot 106: 957-965

Lutmann PJ, Freeman SE, Pekrun C (2003) Long term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. Journal of Agricultural Science 141: 231-240

Lutmann PJ, Berry K, Payne RW, Simpson E, Sweet JB, Champion GT, May MJ, Wightman P, Walker K, Lainsbury M (2005) Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (*Brassica napus*). Proceedings of the Royal Society B 272: 1909-1915

Mag T (2001) Canola Seed and Oil Processing, Canola Council of Canada, Winnipeg, Manitoba.

Matportalen (2013) Allergimerking. http://www.matportalen.no/merking/tema/merking_av_mat/allergimerking

Messéan A, Angevin F, Gomez-Barbero M, Menrad K, Rodriguez-Cerezo E (2006) New case studies on the coexistence of GM and non-GM crops in European agriculture. ISBN: 92-79-01231-2. Technical Report EUR 22102 EN. European Communities 2006

Messéan A, Sausse C, Gasquez J, Darmency H (2007) Occurrence of genetically modified oilseed rape seeds in the harvest of subsequent conventional oilseed rape over time. Eur J Agron 27: 115-122

Mikkelsen TR, Andersen B, Jørgensen RB (1996) The risk of crop transgene spread. Nature 380: 31

Mikkelsen TR, Jørgensen RB (1997) Kulturafgrøders mulige krydsningspartnere i Danmark. Danske dyrkede planters hybridisering med den vilde danske flora. Skov- og Naturstyrelsen og Forskingscenter Risø. København. 84 s.

Miller-Cebert RL, Sistani NA, Cebert E (2009),"Panelists Liking of Canola (Brassica napus) Greens", Nutrition and Food Science Vol.39, No. 6, pp. 627-635.

Moyes CL, Lilley JM, Casais CA, Cole SG, Haeger PD, Dale PJ (2002). Barriers to gene flow from oilseed rape (Brassica napus) into populations of Sinapis arvensis. Molecular Ecology 11: 103-112

NBF (1999) Norsk Botanisk Forening. Årsmeldinger for 1998 og ekskusjonsreferater for sommer/host 1998. Blyttia 57: 62-83

Netherwood T, Martín-Orúe SM, O'Donnell AG, Gockling S, Graham J, Mathers JC, Gilbert HJ (2004) Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nature Biotechnology 22: 204-209

NIAB (2006) Report from the separation distances required to ensure GM content of harvested material from the neighbouring is below specific limits in non-seed crops of oilseed rape. maize and sugar beet. http://www2.defra.gov.uk/

Nielsen KM, van Elsas JD, Smalla K (2000) Transformation of Acinetobacter sp. 13 (pFG4deltanptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Applied Environmental Microbiology 66: 1237-42

Nielsen KM, Townsend JP (2004) Monitoring and modeling horizontal gene transfer. Nature Biotechnology 22(9): 1110-1114

Nishizawa T, Nakajima N, Aono M, Tamaoki M, Kubo A, Saji H (2009) Monitoring the occurrence of genetically modified oilseed rape growing along a Japanese roadside: 3-year observations. Environmental Biosafety Research 8: 33-44

Nishizawa T, Tamaoki M, Aono M, Kubo A, Saji H, Nakajima N (2010) Rapeseed species and environmental concerns related to loss of seeds of genetically modified oilseed rape in Japan. GM Crops 1: 1–14

Norris C, Sweet J (2002) Monitoring large scale releases of genetically modified crops (EPG1/5/84) incorporating report on project EPG 1/5/30: monitoring releases of genetically modified crop plants. DEFRA report, EPG 1/5/84.

Norris C, Sweet J, Parker J, Law J (2004) Implications for hybridisation and introgression between oilseed rape (*Brassica napus)* and wild turnip (*B. rapa*) from an agricultural perspective. In: den Nijs HCM. Bartsch D. Sweet J (eds) Introgression from genetically Modified Plants into Wild relatives. CABI publishing. pp 107-123

OECD (2001) OECD) (2001) Consensus document on key nutrients and key toxicants in low erucic acid rapeseed (canola). Report No. ENV/JM/MONO(2001)13.

OECD (2011) Revised Consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola): Key food and feed nutrients, anti-nutrients and toxicants. Series on safety of novel foods and feeds NO. 24. ENV/JM/MONO(2011)55. Organisation for Economic Co-operation and Development. Paris.

OECD (2012) Consensus document on the biology of the *Brassica* crops (*Brassica* ssp.). Series on Harmonisation of Regulatory Oversight in Biotechnology. ENV/JM/MONO(2012)41.

OGTR (2008) Australian Government. department of Health and Ageing - Office of the Gene Technology Regulator. The biology of *Brassica napus* L. (canola). Version 2.

Orson J (2002) Gene stacking in herbicide tolerant oilseed rape: Lessons from the North American Experience. English Nature Research Reports No 443. pp 17

Pascher K, Macalka S, Rau D, Gollmann G, Reiner H, Glössl J, Grabherr G (2010) Molecular differentiation of commercial varieties and feral populations of oilseed rape (*Brassica napus* L.) Evolutionary Biology 10:63

Pekrun C, Potter TC, Lutman PJW (1997) Genotypic variation in the development of secondary dormancy in oilseed rape and its impact on the persistence of volunteer rape. Proceedings of the 1997 Brighton Crop Protection Conference – Weeds. Brighton. UK (British Crop Protection Council). 243–248.

Pekrun C, Hewitt JDJ, Lutman PJW (1998) Cultural control of volunteer oilseed rape (*Brassica napus*). Journal of Agricultural Science 130: 155-163

Peltzer DA, Ferriss S, FitzJohn RG (2008) Predicting weed distribution at the landscape scale: using naturalised Brassica as a model system. Journal of Applied Ecology 45: 467–475

Pessel FD, Lecomte J, Emeriau V, Krouti M, Messean A, Gouyon PH (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. Theoretical and Applied Genetics 102: 841-846

Pivard S, Adamczyk K, Lecomte J, Lavigne C, Bouvier A, Deville A, Gouyon PH. Huet S (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. Journal of Applied Ecology 45: 476–485

Poikonen S, Puumalainen TJ, Kautianinen H, Burri P, Palosuo T, Reunala T, Turjanmaa K (2006) Turnip rape and oilseed rape are new potential food allergens in children with atopic dermatitis. Allergy 61: 124-127.

Puumalainen TJ, Poikonen S, Kotovuori A, Vaali K, Kalkkinen N, Reunala T, et al. (2006) Napins, 2S albumins, are major allergens in oilseed rape and turnip rape. J Allergy Clin Immunol 117: 426-432.

Rieger M, Lamond M, Preston C, Powles S, Roush R (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. Science 296: 86-88

Rizzi AN, Raddadi C, Sorlini L, Nordgård K, Nielsen KM, Daffonchio D (2012) The stability and degradation of dietary DNA in the gastrointestinal tract of mammals - implications for horizontal gene transfer and the biosafety of GMOs. Crit. Rev. Food Science Nutr. 52: 142-161

Saji H, Nakajima N, Aono M, Tamaoki M, Kubo A, Wakiyama S, Hatase Y, Nagatsu M (2005) Monitoring the escape of transgenic oilseed rape around Japanese ports and roadsides. Environmental Biosafety Research 4: 217-222

Sanvido O, Stark M, Romeis J, Bigler F (2006) Ecological impacts of genetically modified crops. Experiences from ten years of experimental field research and commercial cultivation. ART-Schriftenreihe 1. October 2006. 85 pp.

Schafer MG, Ross AA. Londo JP. Burdick CA. Lee EH. et al. (2011): The Establishment of Genetically Engineered Canola Populations in the U.S. PLoS ONE 6(10): e25736. doi:10.1371/journal.pone.0025736

Scheffler J, Parkinson R, Dale P (1995) Evaluating the effectiveness of isolation distance for field plots of oilseed rape (*Brassica napus*) using a herbicide resistance transgene as a selectable marker. Plant Breeding 114: 317-321

Schubbert GW, Lettmann C, Doerfler W (1994) Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Molecular & General Genetics 242: 495-504

Scott SE, Wilkinson MJ (1998) Transgene Risk is Low. Nature 393: 320

SCP (1998) Opinion of the SCP regarding the Glufosinate tolerant. hybrid rape derived from genetically modified parental lines (MS8xRF3) notified by Plant Genetic Systems (notification C/BE/96/01) Submitted by the Scientific Committee on Plants. 19 May 1998.

Skretting (2010) Skretting Miljørapport. Skretting Norway

SLF (2011) Råvareforbruk av kraftfôr til husdyr i Norge 2010. Statens landbruksforvalting.

SLF (2013) Råvareforbruk av kraftfôr til husdyr i Norge 2012. Statens landbruksforvalting. https://www.slf.dep.no/no/produksjon-og-marked/korn-og-kraftfor/rapportering-salg-og-produksjon/statistikk

Snow AA, Andersen B, Jørgensen RB (1999) Cost of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa.* Molecular Ecology 8: 605-615

Squire GR, Breckling B, Dieter-pfeilstetter A, Jørgensen RB, Lecomte J, Pivard S, Reuter H, Young MW (2011) Status of feral oilseed rape in Europe: its minor role as a GM impurity and its potenstial as a reservoir of transgene persistence. Environ Sci Pollut Res 18:111-115

Statistics Norway (2011). Statistisk Sentralbyrå - Korn og oljevekster. areal og avlinger.

Tamis WLM, de Jong TJ (2009) Transport chains and seed spillage of potential GM crops with wild relatives in the Netherlands. GOGEM report: CGM 2009

Tan SH, Mailer RJ, Blanchard CK, Agboola SO (2011) Canola proteins for human consumption: Extraction, profile, and functional properties. Journal of Food Science Vol 76. Nr 1.

Thompson CE, Squire G, Mackay G. Bradshaw JE, Crawford J, Ramsay G (1999) Regional patterns of gene flow and its consequences for GM oilseed rape. *In:* ' Gene Flow and Agriculture: Relevance for Transgenic Crops. Lutman. P. (ed.). BCPC Symposium Proceedings No. 72.

Timmons AM, O'Brien ET, Charters YM, Dubbels SJ, Wilkinson MJ (1995) Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera.* Euphytica 85: 417-423

Timmons AM, Charters Y, Crawford J, Burn D, Scott S, Dubbels S, Wilson N, Robertson A, O'Brian E, Squire G,Wilkinson M (1996) Risks from transgenic crops. Nature 380: 487

Tolstrup K, Andersen S, Boelt B, Buus M, Gylling M, Holm PB, Kjellson G, Pedersen S, Østergård H, Mikkelsen SA (2003) Report from the Danish working group on the co-existence of genetically modified crops with conventional and organic crops. DIAS report Plant Production no. 94. Fredriksberg Boktryk. Denmark. 275p.

Tolstrup K, Andersen SB, Boelt B, Gylling M, Bach Holm P, Kjellson G, Pedersen S, Østergård H, Mikkelsen SA (2007) Supplementary Report from the Danish Working Group on the Co-existence of genetically Modified Crops with Conventional and Organic Crops. DJF Plant science N.13. 107p.

Treu R, Emberlin J (2000) Pollen dispersal in the crops maize (Zea mays). oil seed rape (*Brassica napus* ssp. *oleifera*). potatoes (*Solanum tuberosum*). sugar beet (*Beta vulgaris* ssp. *vulgaris*) and wheat (*Triticum aestivum*). Evidence from publications. A report for the Soil Assosiation. January 2000.

Vandermarliere N, De Beuckeleer M (2004) Analysis of the presence or absence of bar, barnase-, barstar and ceypyic RNA transcripts in oilseed rape tissues from MS8, RF3 and MS8RF3 hybrids. BioScience NV. 80 s. Technical Dossier from the notifier.

Van der Klis RJ (2002) PAT ELISA on different fractions from the industrial processing of MS8RF3 winter oilseed rape seed (Brassica napus). Avensis CropSciences NV. 14 s.

Van der Klis RJ (2004) Analysis of the presence or absence of PAT, Barnase, Barstar and Barnase/Barstar protein complex in oilseed rape tissues from MS8, RF3 and MS8RF3 hybrids. Bayer CropScience NV. 33 s.

VKM (2005) Report from an Ad Hoc Group appointed by the Norwegian Scientific Panel on Genetically Modified Organisms and Panel on Biological Hazards – An assessment on potentially long-term health effects caused by antibiotic resistance marker genes in genetically modified organisms based on antibiotic usage and resistance patterns in Norway. Opinion 05/302-1-final. Norwegian Scientific Committee for Food Safety. Oslo. Norway 62 p.

VKM (2007) Vurdering av foreslåtte virkemidler for sameksistens mellom genmodifiserte vekster og konvensjonelt/økologisk landbruk. og rangering av spredningsrisiko av transgener fra relevante genmodifiserte planter som kan dyrkes i Norge. Uttalelse fra Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet. 06/305-endelig. Vitenskapskomiteen for mattrygghet. Oslo. Norge.

VKM (2008) Helse- og miljørisikovurdering av genmodifisert oljeraps. linje MS8. RF3 og MS8 x RF3 fra Bayer CropScience AG (C/BE/96/01). Uttalelse fra Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet 24.6.2008. 08/307-endelig. Vitenskapskomiteen for mattrygghet. Oslo. Norge.

VKM (2012) Helse- og miljørisikovurdering av genmodifisert raps MS8. RF3. MS8 x RF3 (EFSA/GMO/BE/2011/81). Uttalelse fra Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet. 11/312- utkast. Unpublished.

Warwick SI (2004) Gene flow between canola varieties and to other wild species *In:* Sameksistens (Coexistence). Handouts. Seminar by The Norwegian Biotechnology Advisory Board.

Warwick SI, Simard MJ, Legere A, Beckie HJ, Braun L, Zhu B, Mason P, Seguin-Swartz G, Stewart CN (2003) Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L. and *Erucastrum gallicum* (Willd.) O.E. Schulz. Theoretical and Applied Genetics 107:528-539

Warwick SI, Légère A, Simard MJ, James T (2008) Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. Molecular Ecology 17: 1387-1395

Weekes R, Deppe C, Allnutt T, Boffey C, Morgan S, Bilton M, Daniels R. Henry C (2005) Crop-to-crop gene flow using farm scale sites of oilseed rape (*Brassica napus*) in the UK. Transgenic Res 14:749-759

Weston B (1998) Evaluation of the stability of MS8, RF3, MS8xRF3 in Brassica napus cv. Drakkar (SOSR) under different environmental conditions - Plant Genetic Systems N.V. - 27 pages - # M-147986-01-1.

Wilkinson MJ, Davenport IJ, Charters YM, Jones AE, Allainguillaume J, Butler HT, Mason DC. Raybould AF (2000) A Direct Regional Scale Estimate of Transgene Movement from Genetically Modified Oilseed Rape to its Wild Progenitors. Mol Ecol 9: 983-991

Xiao L, Lu C et al. (2009) Gene transferability from transgenic *Brassica napus* L. to various subspecies and varieties of *Brassica rapa.* Transgenic Research 18: 733-746

Yoshimura Y, Beckie HJ, Matsuo K (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environmental Biosafety Res 5: 67-75

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

Principal growth stage 7: Development of fruit	
71 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, 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.

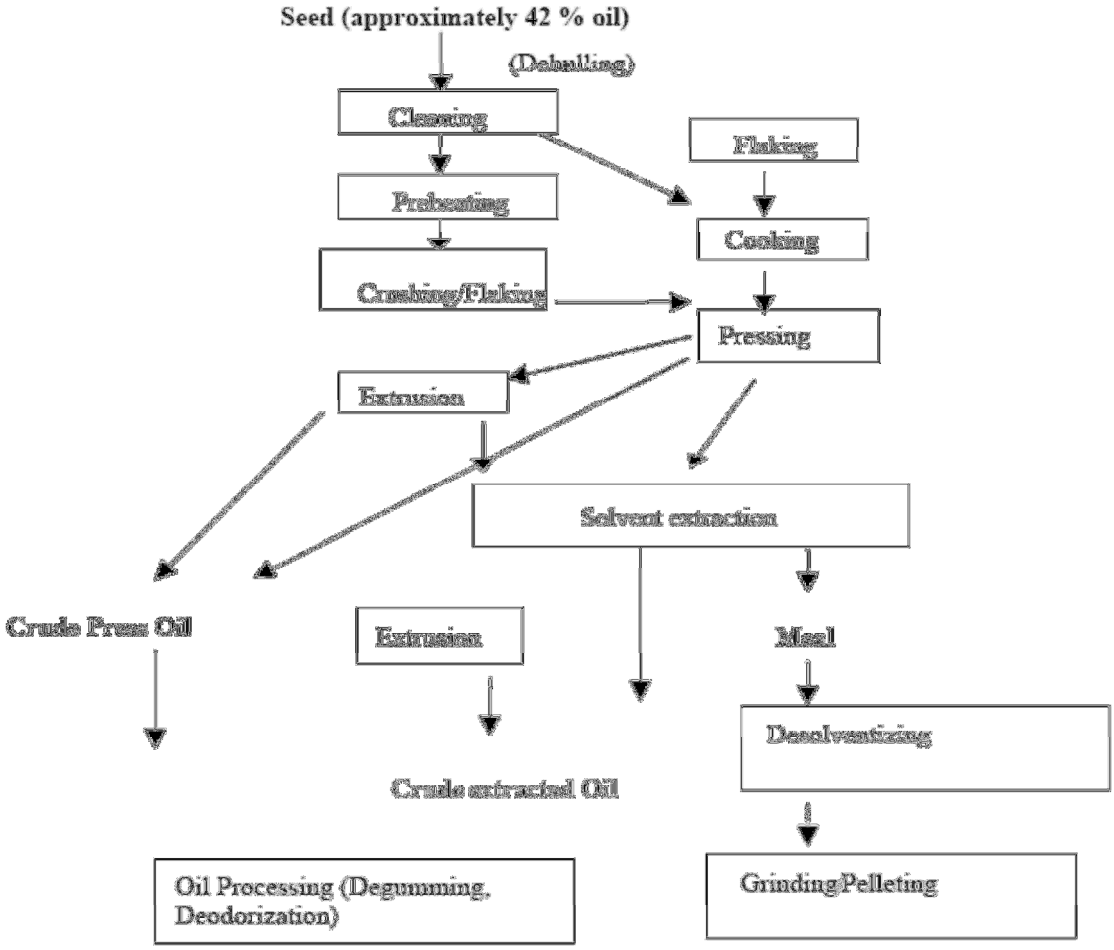


Figure 1. **Schematic illustration of the processing of low erucic acid rapeseed meal and low erucic acid rapeseed oil (OECD 2001).**

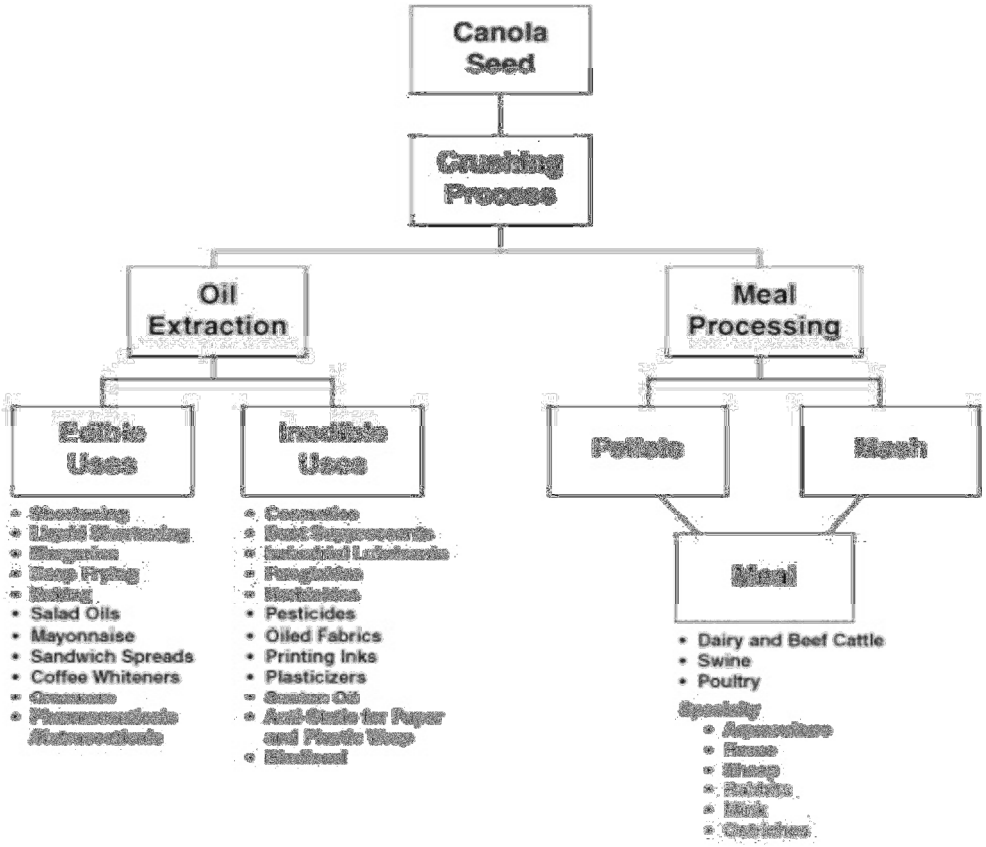


Figure 2 **Areas of application and products from processing of rapeseed (Canola Council of Canada 2005).**

Appendix 2

Table 1. Proximates in SeedLink® (SL) oilseed rape (OSR) Event MS8 x RF3 Seeds, Non-transgenic Counterpart (reference variety Drakkar) and Commercial OSR Varieties grown in Europe in the growth seasons 2001 and 2002 (Oberdörfer, 2003)

	Non-Transgenic	Transgenic Not sprayed	Transgenic Sprayed	Standard-Values [a]
Moisture %fw	5,17 ± 0,74	5,24 ± 0,65	5,28 ± 0,64	5,4 - 17,0
Fat %dm	45,97 ± 1,87	46,23 ± 1,77	46,45 ± 1,88	24,0 - 52,6
Protein %dm	19,85 ± 2,34	20,01 ± 2,37	19,87 ± 2,38	18,7 - 26,0
Ash %dm	4,46 ± 0,31	4,47 ± 0,35	4,43 ± 0,26	4,1 - 5,0
Total Carbohydrates %dm[d]	29,71 ± 1,02	29,30 ± 0,94	29,25 ± 0,92	25,4 - 53,2
ADF %dm	14,06 ± 2,22	14,51 ± 1,83	14,20 ± 1,95	**10,0 - 10,8**
NDF %dm	30,85 ± 6,05	27,69 ± 5,12	28,04 ± 4,23	**16,0 - 18,5**

[a] Standard values from table 2 of appendix A.
[b] Summary of the equivalence evaluation non-transgenic versus transgenic, not Liberty® treated, over all sites
[c] Summary of the equivalence evaluation non-transgenic versus transgenic, Liberty® treated, over all sites
[d] Total Carbohydrates calculated as 100% - (protein %dm + fat %dm+ ash %dm)

Table 2. Minerals in SL OSR Event MS8 x RF3 Seeds, Non-trensgenic Counterpart and Commercial OSR Varieties grown in Europe in the growth seasons 2001 and 2002 (Oberdörfer, 2003)

Parameter	On dry matter basis			
	Non-Transgenic	Transgenic Not sprayed	Transgenic Sprayed	Standard-Values [a]
Calcium mg/kg	**5468 ± 413**	**5674 ± 458**	**5615 ± 496**	2900 - 4800 [d]
Phosphorus mg/kg	8179 ± 844	8445 ± 869	8375 ± 833	4800 - 8500 [d]
Potassium mg/kg	**7776 ± 721**	**7699 ± 667**	**7609 ± 598**	8300 - 9100 [d]
Magnesium mg/kg	**2721 ± 312**	**2744 ± 297**	**2713 ± 258**	2900 - 3100 [d]
Sodium mg/kg	<100 – 176	<100 - 212	<100 - 153	100 - 900 [d]
Iron mg/kg	**68,59 ± 10,03**	**66,65 ± 6,90**	**64,89 ± 6,84**	160 - 640 [e]
Manganese mg/kg	**35,20 ± 6,24**	**35,52 ± 7,28**	**35,70 ± 7,65**	43 - 73 [e]
Copper mg/kg	**3,84 ± 0,33**	**3,87 ± 0,75**	**3,81 ± 0,51**	4 - 11,3 [e]
Zinc mg/kg	43,17 ± 7,28	41,93 ± 6,33	42,36 ± 7,17	43 - 77,6 [e]

[a] Standard values from table 2 and 5 of appendix A
[b] Summary of the equivalence evaluation non-transgenic versus transgenic, not Liberty® treated, over all Sites
[c] Summary of the equivalence evaluation non-transgenic versus transgenic, Liberty® treated, over all sites
[d] conversion factor (f) from %dm to mg/kg dm f = 10 000
[e] only values for rapeseed meal or values for rapeseed meal included
[f] values were not obtained by calculation of the mean, since all results are below LOQ.
[g] sum of alpha and gamma tocopherol

Table 3. Tocopherols in SL OSR Event MS8 x RF3Seeds, Non-transgenic Counterpart and Commercial OSR Varieties grown in Europe in the growth seasons 2001 and 2002 (Oberdörfer, 2003)

Parameter	On dry matter basis			
	Non-Transgenic	Transgenic Not sprayed	Transgenic Sprayed	Standard-Values [a]
Alpha Tocopherol mg/100g	13,75 ± 1,30	12,88 ± 0,76	12,90 ± 0,78	4,5 - 17,4
Beta Tocopherol mg/100g	< 1,00 [f]	< 1,00 [f]	< 1,00 [f]	0 - 6,3
Gamma Tocopherol mg/100g	16,79 ± 2,00	16,82 ± 1,14	16,91 ± 1,19	8,5 - 21,9
Delta Tocopherol mg/100g	< 1,00 [f]	< 1,00 [f]	< 1,00 [f]	0 - 1,0
Total Tocopherols mg/100g [g]	30,6	29,7	29,8	19,4 - 120,5

[a] Standard values from table 2 and 5 of appendix A

Table 4. Anti-nutrients in SL OSR Event MS8 x RF3 Seeds, Non-transgenic Counterpart Drakkar and Commercial OSR Varieties grown in Europe in the growth seasons 2001 and 2002 (Oberdörfer, 2003)

Parameter	On seed dry matter basis			
	Non-Transgenic	Transgenic Not sprayed	Transgenic Sprayed	Standard-Values [a]
Phytic acid %	2,04 ± 0,29	2,08 ± 0,29	2,09 ± 0,30	2,0 - 5,0
Alkenyl glucosinolate µmol/g	11,40 ± 2,94	13,48 ± 3,11	12,91 ± 2,99	ND
Aromatic glucosinolate µmol/g	<LOQ – 0,50	<LOQ – 0,36	<LOQ – 0,50	ND
Indolyl glucosinolate µmol/g	2,13 ± 1,30	2,22 ± 1,45	2,14 ± 1,26	ND
Total glucosinolate µmol/g	13,63 ± 3,29	15,81 ± 3,44	15,15 ± 3,36	3,8-33,9

ND no data available
[a] Standard values from table 7 of appendix A
[b] Summary of the equivalence evaluation non-transgenic versus transgenic, not Liberty®treated, over all sites
[c] Summary of the equivalence evaluation non-transgenic versus transgenic, Liberty®treated, over all sites

Table 5. Total Amino Acids in SL OSR Event MS8 x RF3 Seeds. Non-transgenic Drakkar and Commercial OSR Varieties grown in Europe in the growth seasons 2001 and 2002 (Oberdörfer, 2003)

Parameter	mg/g Dry matter			
	Non-Transgenic	Transgenic Not sprayed	Transgenic Sprayed	Standard-Values [a,b]
Alanine	8,87 ± 1,11	8,87 ± 1,08	8,82 ± 1,08	9,3 – 9,6
Arginine	12,09 ± 1,59	12,30 ± 1,61	12,19 ± 1,56	11,3 – 12,1
Aspartic acid	**15,59 ± 2,05**	**15,17 ± 1,95**	**15,11 ± 1,94**	32,9[e]
Cystine	4,59 ± 0,54	4,89 ± 0,59	4,79 ± 0,58	5,2 – 5,4
Glutamic acid	**33,76 ± 5,25**	**34,84 ± 5,47**	**34,45 ± 5,23**	70,2 [e]
Glycine	10,36 ± 1,23	10,35 ± 1,20	10,26 ± 1,16	10,4 – 10,6
Histidine	5,42 ± 0,63	5,59 ± 0,68	5,53 ± 0,63	5,1 – 6,6
Isoleucine	7,89 ± 0,99	8,09 ± 1,12	8,04 ± 1,07	8,0 – 8,6
Leucine	13,87 ± 1,68	14,02 ± 1,76	13,92 ± 1,67	13,5 – 14,7
Lysine	12,06 ± 1,20	12,35 ± 1,29	12,24 ± 1,32	10,3 – 11,9
Methionine	3,92 ± 0,39	4,05 ± 0,43	4,03 ± 0,45	4,2 – 4,4
Phenylalanine	7,96 ± 1,04	8,07 ± 1,13	8,00 ± 1,07	7,5 – 8,2
Proline	11,59 ± 1,36	12,00 ± 1,39	11,96 ± 1,37	11,9 – 13,3
Serine	9,23 ± 1,07	9,02 ± 0,91	8,98 ± 0,92	9,0 – 9,4
Threonine	8,47 ± 1,00	8,40 ± 0,94	8,35 ± 0,93	8,7 – 9,4
Tryptophan	2,10 ± 0,29	2,12 ± 0,30	2,09 ± 0,31	2,3 – 2,7
Tyrosine	5,59 ± 0,65	5,62 ± 0,68	5,59 ± 0,65	5,1 – 5,9
Valine	10,37 ± 1,26	10,55 ± 1,39	10,44 ± 1,30	10,2 – 11,3

[a] Standard values from table 3 of appendix A.
[b] Conversion factor from % dm to mg/g dm f = 10
[c] Summary of the equivalence evaluation non-transgenic versus transgenic, not Liberty®treated, over all sites
[d] Summary of the equivalence evaluation non-transgenic versus transgenic, Liberty®treated, over all sites
[e] values were only available for rapeseed meal

Table 6. Total Fatty acids in SL OSR Event MS8 x RF3 Seeds, Non-transgenic Counterpart and Commercial OSR Varieties (in % of sum of total fatty acids) grown in Europe in the growth seasons 2001 and 2002 (Oberdörfer, 2003)

		% Relative			
Fatty Acid		**Non-transgenic**	**Transgenic Not sprayed**	**Transgenic Sprayed**	**Std. Values[a]**
Saturated	Palmitic C16:0	4,84	4,59	4,58	1,5-7,0
	StearicC18:0	1,74	1,73	1,74	0,5-3,1
	Arachidic C20:0	0,66	0,65	0,64	0-3,0
	Behenic C22:0	0,41	0,42	0,39	0-2,0
	Lignoceric C24:0	< 0,2	< 0,2	< 0,2	0-2,0
Total Saturated		*7,66*	*7,39*	*7,35*	*6,0-7,1*
Mono-unsaturated	Palmitoleic C16:1	0,25	0,25	0,25	0-3,0
	Heptadecenoic C17:1	< 0,2	< 0,2	< 0,2	ND
	Oleic C18:1	62,26	62,70	62,90	8,0-70,0
	Eicosenoic C20:1	1,24	1,22	1,21	0,1-15,0
Total Mono-uns.		*63,75*	*64,18*	*64,37*	*58,9-61,9*
Poly-unsaturated	Linoleic C18:2	18,67	18,42	18,27	11,0-30,0
	Linolenic C18:3	9,93	10,02	10,00	5,0-14,8
	Docosapentaenoic C22:5	< 0,2	< 0,2	< 0,2	ND
	Docohexaenoic C22:6	< 0,2	< 0,2	< 0,2	ND
Total Poly-uns.		*28,59*	*28,43*	*28,28*	*29-30*
Sum of total fatty acids		*100*	*100*	*100*	*95,6-97,6*

ND no data
a Standard values from table 4 of appendix A.

Table 7 .Total Fatty acids in SL oilseed rape Event MS8 x RF3 seeds, non-transgenic counterpart "Drakkar" (in % of sum of total fatty acids) grown in Europe in the growth seasons 2001 and 2002 (Oberdörfer, 2003)

Components measured	Non-transgenic counterpart «Drakkar»		MS8 x RF3 Not glufosinate treated		MS8 x RF3 Glufosinate treated		Codex 2005 Standard variation LEAR
	Mean±SD, % dm	Mean % total fatty acids	Mean ± SD, % dm	Mean % total fatty acids	Mean ± SD, % dm	Mean % total fatty acids	% of total fatty acids
Saturated fatty acids							
Palmitic C16:0	2.11±0.11	4.84	1.99±0.11	4.59	2.0 ± 0.12	4.58	2.5-7.0
Stearic C18:0	0.76±0.05	1.74	0.75±0.05	1.73	0.76± 0.06	1.74	0.8-3.0
Arachidic C20:0	0.29±0.02	0.66	0.28±0.02	0.65	0.28±0.03	0.64	0.2-1.2
Behenic C22:0	0.18±0.02	0.41	0.18±0.02	0.42	0.17±0.03	0.39	ND-0.6
Lignoceric C24:0	<0.05-0.07	<0.2	<0.05-0.07	<0.2	<0.05-0.07	<0.2	ND-0.3
Mono unsaturated fatty acids							
Palmitoleic C16:1	0.11±0.01	0.25	0.11±0.01	0.25	0.11±0.01	0.25	ND-0.6
Oleic C18:1	27.15± 1.24	62.26	27.17±1.27	62.70	27.47±1.57	62.90	51.0-70.0
Gadoleic (Eicosenoic) C20:1	0.54±0.03	1.24	0.53±0.03	1.22	0.53±0.03	1.21	0.1-4.3

Poly unsaturated fatty acids							
Linoleic C18:2	8.14± 0.30	18.67	7.98±0.39	18.42	7.98±0.44	18.27	15.0-30.0
Linolenic C18:3	4.33±0.32	9.93	4.34±0.32	10.02	4.37±0.34	10.00	5.0-14.0

ND -no data; a) Standard values for fatty acids (% of total fatty acids) in oil from oilseed rape. (Standard values are the literature range from different sources. i.e. OECD. USDA etc.).

Tabell 8. Overview of mean, standard deviation (SD) and reference range of proximate and fibre compounds in seed of MS8, RF3, MS8 x RF3 and non-transgenic negative segregant oilseed rape grown at five locations in Canada during the 2008 growing season (Oberdörfer 2009a,b).

Components measured	Non-transgenic negative segregant [a]		Non-transgenic negative segregant[b]		MS8[c]		RF3[d]		MS8 x RF3[e]		Reference range[f]
	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean±SD	Min-max	Mean± SD	Min-max	Mean±SD	Min-max	Min-max
Moisture (% fw)	5.81±0.26	5.40-6.37	5.72±0.28	5.13-6.12	5.73±0.25	5.31-6.22	5.58±0.32	4.99-6.09	5.88± 0.22	5.59-6.36	5.19-6.26
Crude fat (% dm)	41.3±3.1	35.9-47.0	43.1±2.2	39.4-47.2	42.1±2.6	36.6-47.4	43.5±2.6	37.3-48.4	42.2± 3.0	38.0-48.8	37.7-47.5
Crude protein (% dm)	27.1±2.0	23.1-30.5	26.1±2.2	21.8-29.8	28.1±2.5	22.7-31.7	25.1±2.8	19.9-29.4	26.1± 2.3	21.5-29.8	21.1-29.6
Ash (% dm)	3.99±0.2	3.53-4.46	3.79±0.22	3.27-4.15	3.99±0.21	3.63-4.44	3.81±0.22	3.43-4.18	3.92± 0.22	3.54-4.33	3.61-4.12
Total Carb (% dm)	27.7±1.7	24.2-30.7	27.0±1.6	24.0-29.4	25.8±1.1	24.3-28.0	27.5±2.1	24.5-33.1	27.8± 1.8	25.4-32.5	26.1-31.2
ADF (% dm)	34.7±2.3	30.8-39.9	34.8±3.2	29.2-40.5	32.7±2.8	28.5-38.1	35.6±2.5	29.7-40.6	36.0± 2.9	31.1-40.3	28.9-42.1
NDF (% dm)	36.7±2.7	31.1-41.7	38.4±3.5	33.7-44.1	35.5±2.7	28.9-42.7	39.3±2.1	34.8-44.4	38.7±2.6	33.9-42.4	30.2-44.5

[a] Non-transgenic negative segregant oilseed rape with a "female of hybrid A" genetic background – conventionally treated.
[b] Non-transgenic negative segregant oilseed rape with a "male of hybrid A" genetic background – conventionally treated
[c] MS8 oilseed rape with a "female of hybrid A" genetic background – glufosinate treated.
[d] RF3 oilseed rape with a "male of hybrid A" genetic background – glufosinate treated. [e] MS8xRF3 oilseed rape with a "hybrid A" genetic background – glufosinate treated.
[f] Reference range from literature data.

Tabell 9. Overview of mean, standard deviation (SD) and reference range of minerals in seed of MS8, RF3, MS8 x RF3 and non-transgenic negative segregant oilseed rape grown at five locations in Canada during the 2008 growing season (Oberdörfer 2009a.b).

Components measured	Non-transgenic negative segregant[a]		Non-transgenic negative segregant[b]		MS8[c]		RF3[d]		MS8 x RF3[e]		Reference range
	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean± SD	Min-max	Min-max
Calsium (%)	0.369 ±0.04	0.300 - 0.424	0.412 ±0.025	0.357-0.450	0.358 ±0.033	0.293 - 0.403	0.412 ±0.022	0.368 0.449	0.395± 0.034	0.341-0.463	0.29-0.48
Phosphorus (%)	0.686 ±0.053	0.587 - 0.762	0.648 ±0.052	0.554-0.732	0.706 ±0.064	0.600 - 0.842	0.622 ±0.053	0.550 - 0.722	0.649± 0.057	0.543-0.742	0.48-0.85
Potassium (%)	0.779 ±0.082	0.612 - 0.901	0.689 ±0.058	0.589-0.786	0.749 ±0.052	0.655 - 0.835	0.677 ±0.046	0.594 - 0.735	0.727± 0.076	0.586-0.803	0.83-0.91
Magnesium (%)	0.314 ±0.013	0.294 - 0.340	0.304 ±0.017	0.280-0.338	0.312 ±0.018	0.282 - 0.350	0.294 ±0.013	0.270 - 0.312	0.308± 0.014	0.286-0.336	0.29-0.31
Sodium (%)	<0.0100-0.0190		<0.0100-0.0154		<0.0100-0.0201		<0.0100-0.0107		<0.0100-0.0153		0.05
Iron (mg/kg)	69.7± 10.7	59.2-105.0	61.0± 3.8	56.6-70.4	69.4 ±6.1	62.7-84.8	61.7 ±11.9	50.5-90.5	68.8± 12.6	55.6-110.0	ND
Manganese (mg/kg)	43.7± 3.2	37.6-49.7	42.7± 2.3	39.4-47.2	41.7 ±2.7	37.4-46.5	41.8 ±1.3	38.9-43.9	44.7±1.9	40.3±47.3	ND
Copper (mg/kg)	3.56± 0.23	3.11-4.08	3.58± 0.27	2.99-4.04	3.84 ±0.23	3.22-4.16	3.33 ±0.22	2.89-3.75	3.17±0.35	2.59±4.01	7.0
Zink (mg/kg)	57.5± 5.6	48.3-66.7	41.4± 2.4	36.7-46.1	58.3 ±5.2	51.1-66.6	40.1 ±2.9	34.3-45.5	47.8±4.3	41.8-55.4	62
Alfa-tocopherol (mg/kg)	101.8 ±11.0	83.3-120.0	73.3± 10.6	56.2-92.3	96.8 ±10.2	78.2-117.0	68.9 ±9.5	55.5-87.3	92.6±10.2	73.9-114	24-203
Beta-tocopherol (mg/kg)	<5.00		<5.00		<5.00		<5.00		<5.00		0-74
Gamma-tocopherol (mg/kg)	212.1 ±12.0	193.0 - 241.0	245.2 ±27.4	175.0-280.0	195.8±19.6	159.0-231.0	237.5±26.7	186.0-287.0	213.8±19.3	175.0-242.0	45-396

Delta-tocopherol (mg/kg)	<5.00-8.99		<5.00-8.81		<5.00-9.86		<5.00-9.48		<5.00-11.1		0-12
Total tocopherol (mg/kg)	320.6 ±22.1	276-362	324.5 ±38.4	232-368	299.6±29.0	243-352	312.4±37.4	242-381	312.9±28.3	250-357	94-1410

[a] Non-transgenic negative segregant oilseed rape with a "female of hybrid A" genetic background – conventionally treated.
[b] Non-transgenic negative segregant oilseed rape with a "male of hybrid A" genetic background – conventionally treated
[c] MS8 oilseed rape with a "female of hybrid A" genetic background – glufosinate treated.
[d] RF3 oilseed rape with a "male of hybrid A" genetic background – glufosinate treated. [e] MS8xRF3 oilseed rape with a "hybrid A" genetic background – glufosinate treated.
[f] Reference range from literature data.

Table 10. Overview of mean, standard deviation (SD) and reference range of amino acids in seed of MS8, RF3, MS8 x RF3 and non-transgenic negative segregant oilseed rape grown at five locations in Canada during the 2008 growing season (Oberdörfer 2009a.b).

Komponenter analysert (% TS)	Non-transgenic negativ segregant[a]		Non-transgenic negativ segregant[b]		MS8[c]		RF3[d]		MS8 x RF3[e]		Reference range
	Mean±SD	Min-max	Mean± SD	Min-max	Mean±SD	Min-max	Mean± SD	Min-max	Mean±SD	Min-max	Min-max
Alanine	1.19±0.08	1.10-1.33	1.14±0.10	0.98-1.31	1.22±0.10	1.02-1.36	1.12±0.11	0.92-1.29	1.14±0.09	0.93-1.30	0.93-0.96
Arginine	1.88±0.14	1.58-2.13	1.78±0.18	1.49-2.07	1.94±0.18	1.56-2.22	1.75±0.21	1.40-2.06	1.80±0.17	1.43-2.09	1.13-1.21
Aspartic acid	1.88±0.14	1.56-2.12	1.83±0.19	1.51-2.12	1.93±0.18	1.57-2.21	1.80±0.22	1.41-2.13	1.85±0.18	1.47-2.14	ND
Cystine	0.71±0.05	0.64-0.81	0.68±0.06	0.55-0.77	0.74±0.07	0.62-0.84	0.66±0.07	0.53-0.76	0.67±0.07	0.55-0.77	0.52-0.54
Glutamic acid	5.07±0.04	4.21-5.85	4.86±0.49	4.07-5.58	5.28±0.51	4.21-6.04	4.73±0.54	3.78-5.53	4.84±0.47	3.84-5.62	ND
Glycine	1.39±0.10	1.18-1.57	1.32±0.12	1.11-1.51	1.42±0.12	1.18-1.60	1.30±0.14	1.05-1.49	1.34±0.12	1.08-1.53	1.04-1.06
Histidine	0.77±0.06	0.66-0.87	0.72±0.06	0.62-0.82	0.79±0.07	0.66-0.89	0.71±0.07	0.58-0.81	0.73±0.06	0.60-0.85	0.51-0.66
Isoleucine	1.10±0.08	0.91-1.25	1.08±0.11	0.90-1.27	1.15±0.10	0.97-1.30	1.06±0.12	0.85-1.27	1.07±0.10	0.84-1.24	0.80-0.86
Leucine	1.94±0.14	1.63-2.19	1.85±0.18	1.56-2.16	2.00±0.18	1.65-2.28	1.82±0.21	1.46-2.14	1.85±0.17	1.49-2.14	1.35-1.47
Lysine	1.65±0.11	1.42-1.83	1.60±0.11	1.40-1.77	1.68±0.13	1.42-1.87	1.56±0.14	1.32-1.75	1.59±0.12	1.33-1.79	1.03-1.19

Methionine	0.54±0.04	0.47-0.62	0.54±0.04	0.44-0.60	0.57±0.05	0.46-0.65	0.52±0.06	0.41-0.61	0.53±0.05	0.45-0.60	0.42-0.44
Phenylalanine	1.10±0.08	0.95-1.23	1.07±0.10	0.91-1.25	1.15±0.10	0.95-1.31	1.06±0.11	0.86-1.24	1.07±0.10	0.86-1.23	0.75-0.82
Proline	1.62±0.11	1.41-1.81	1.63±0.17	1.37-1.89	0.71±0.19	1.34-2.02	1.57±0.19	1.27-1.90	1.58±0.14	1.28-1.78	1.19-1.33
Serine	1.14±0.07	0.99-1.29	1.09±0.09	0.95-1.22	1.17±0.11	0.92-1.31	1.07±0.10	0.87-1.21	1.10±0.09	0.93-1.26	0.90-0.94
Threonine	1.14±0.07	1.00-1.28	1.09±0.09	0.95-1.22	1.16±0.09	0.97-1.29	1.08±0.10	0.90-1.21	1.11±0.08	0.95-1.26	0.87-0.94
Tryptophan	0.28±0.03	0.23-0.33	0.27±0.03	0.20-0.33	0.30±0.03	0.24-0.35	0.27±0.04	0.20-0.33	0.27±0.03	0.22-0.33	0.23-0.27
Tyrosine	0.76±0.05	0.67-0.85	0.73±0.06	0.62-0.81	0.78±0.06	0.65-0.87	0.72±0.07	0.59-0.82	0.73±0.06	0.61-0.85	0.51-0.59
Valine	1.39±0.10	1.15-1.53	1.37±0.13	1.15-1.59	1.44±0.12	1.21-1.62	1.34±0.15	1.08-1.59	1.36±0.12	1.08-1.54	1.02-1.13

[a] Non-transgenic negative segregant oilseed rape with a “female of hybrid A” genetic background – conventionally treated
[b] Non-transgenic negative segregant oilseed rape with a “male of hybrid A” genetic background – conventionally treated
[c] MS8 oilseed rape with a “female of hybrid A” genetic background – glufosinate treated.
[d] RF3 oilseed rape with a “male of hybrid A” genetic background – glufosinate treated.
[e] MS8xRF3 oilseed rape with a “hybrid A” genetic background – glufosinate treated.
[f] Reference range from literature data.

Table 11. Overview of mean, standard deviation (SD) and reference range of anti-nutrients in seeds of MS8, RF3 and MS8xRF3 and non-transgenic negative segregant oilseed rape grown at five locations in Canada during the 2008 growing season (Oberdörfer 2009a.b).

Komponenter analysert	Non-transgenic negative segregant[a]		Non-transgenic negativ segregant[b]		MS8[c]		RF3[d]		MS8 x RF3[e]		Reference range
	Mean±SD	Min-max	Mean± SD	Min-max	Mean± SD	Min-max	Mean± SD	Min-max	Mean±SD	Min-max	Min-max
Phytic acid (%TV)	1.85±0.23	1.35-2.14	1.81±0.24	1.43-2.26	1.96±0.2	1.50-2.32	1.73±0.2	1.33± 2.19	1.72±0.2	1.30-1.99	ND
Alkenyl GSL[h] (µmol/g)	9.90±2.93	5.16-16.21	4.61±1.23	2.78-7.16	10.63± 2.67	6.20-14.92	3.98±1.31	2.52-6.71	5.17±1.37	3.54-7.85	ND
MSGL GSL (µmol/g)	<0.05-0.37		<0.05-0.16		0.30±0.12	0.12-0.45	<0.05-0.35		<0.05-0.26		ND
Indole GSL (µmol/g)	6.31±1.02	2.71-7.36	4.75±0.77	3.78-7.48	6.40±1.02	2.73-7.71	5.02± 0.66	3.97-6.70	5.59±0.42	4.58-6.27	ND
Total GSL (µmol/g)	16.42±2.96	10.73-21.82	9.43±1.57	7.31-14.14	17.33± 2.79	12.81-22.55	9.06±1.6	7.01-12.95	10.81± 1.53	8.9-14.0	3.8-33.9

[a] Non-transgenic negative segregant oilseed rape with a "female of hybrid A" genetic background – conventionally treated
[b] Non-transgenic negative segregant oilseed rape with a "male of hybrid A" genetic background – conventionally treated
[c] MS8 oilseed rape with a "female of hybrid A" genetic background – glufosinate treated.
[d] RF3 oilseed rape with a "male of hybrid A" genetic background – glufosinate treated.
[e] MS8xRF3 oilseed rape with a "hybrid A" genetic background – glufosinate treated.
[f] Reference range from literature data.

Table 12. Overview of mean, standard deviation (SD) and reference range of fatty acids in seeds of MS8, RF3 and MS8xRF3 and non-transgenic negative segregant oilseed rape grown at five locations in Canada during the 2008 growing season (Oberdörfer 2009a.b).

Components measured	Non-transgenic negativ segregant[a]		Non-transgenic negativ segregant[b]		MS8[c]		RF3[d]		MS8 x RF3[e]		Reference range
	Mean±SD	Min-max	Mean± SD	Min-max	Mean±SD	Min-max	Mean± SD	Min-max	Mean±SD	Min-max	Min-max
Saturated fatty acids											
Myristic C14:0	0.06±0	0.05-0.06	0.06±0	0.05-0.06	0.05±0	0.05-0.06	0.06±0	0.05±0.07	0.06±0	0.05-0.07	0-0.2
Palmitic C16:0	3.98±0.19	3.72-4.39	4.37±0.14	4.17-4.64	3.96±0.11	3.79-4.27	4.35±0.11	4.10±4.51	4.14±0.16	3.87-4.47	2.5-7.0
Stearic C18:0	2.26±0.2	1.89-2.48	1.96±0.21	1.68-2.41	20.8±0.16	1.82-2.34	1.95±0.24	1.59-2.36	2.21±0.21	1.79-2.50	0.8-3.0
Arachidic C20:0	0.77±0.05	0.68-0.86	0.64±0.04	0.57-0.69	0.72±0.04	0.65-0.80	0.64±0.04	0.57-0.70	0.73±0.05	0.65-0.83	0.2-1.9
Behenic C22:0	0.42±0.03	0.35-0.46	0.33±0.02	0.29-0.37	0.40±0.03	0.34-0.43	0.32±0.02	0.29-0.36	0.38±0.03	0.34-0.42	0-0.6
Lignoceric C24:0	0.22±0.03	0.15-0.30	0.18±0.03	0.13-0.24	0.22±0.02	0.17-0.28	0.19±0.02	0.14-0.23	0.21±0.03	0.16-0.25	0-0.8
Mono unsaturated fatty acids											
PalmitoleicC16:1	0.27±0.01	0.24-0.31	0.26±0.01	0.24-0.28	0.26±0.02	0.24-0.29	0.26±0.01	0.23-0.28	0.26±0.02	0.23-0.29	0-0.6
Oleic C18:1	64.53± 1.18	61.71-66.59	61.00±1.39	59.28-63.50	62.88±1.05	60.93-64.42	60.97± 1.74	57.64-63.71	63.79±1.3	60.92-65.42	50.1-70.0
Gadoleic C20:1	1.29±0.07	1.19-1.44	1.35±0.06	1.27-1.44	1.37±0.07	1.24-1.47	1.35±0.07	1.26-1.51	1.32±0.07	1.22-1.45	0.1-10.9
Erucic C22:1	<0.01-0.03		<0.01-0.03		<0.01-0.19		<0.01-0.03		<0.01-0.03		0-2.0
Nervonic C24:1	0.18±0.04	0.14-0.29	0.15-0.03	0.08-0.20	0.19±0.03	0.13-0.25	0.17±0.04	0.13-0.26	0.16±0.03	0.11-0.20	0-0.4

Poly usaturated fatty acids											
Linoleic C18:2	16.71±0.81	15.60-18.54	18.17±0.55	17.27-19.22	16.97±0.63	16.07-18.35	18.34±0.63	17.06-19.19	17.25±0.66	16.17-18.82	15.0-30.0
Linolenic C18:3	8.74±1.23	7.34-11.25	10.92±1.16	9.22-12.96	10.28±0.72	9.25-11.83	10.81±1.34	9.00-13.34	8.91±1.29	7.72-11.82	5.0-14.8
Eicosadienoic C20:2	0.06±0	0.05-0.07	0.07±0.01	0.06-0.08	0.06±0	0.05-0.07	0.07±0.01	0.06-0.08	0.06±0	0.06-0.07	0-0.1

a Non-transgenic negative segregant oilseed rape with a “female of hybrid A” genetic background – conventionally treated

b Non-transgenic negative segregant oilseed rape with a “male of hybrid A” genetic background – conventionally treated

c MS8 oilseed rape with a “female of hybrid A” genetic background – glufosinate treated.

d RF3 oilseed rape with a “male of hybrid A” genetic background – glufosinate treated.

e MS8xRF3 oilseed rape with a “hybrid A” genetic background – glufosinate treated.

f Reference range from literature data.a Non-transgenic . negativ segregant. med genetisk bakgrunn -hunnplante. hybrid A – konvensjonelt sprøyteprogram

Table 13. 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 14. 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 15. 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 16. 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

Appendix 3

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.

Table 1. 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 an d/or gene introgression: H=High, L=Low, VL=Very low, EL= Extremely low