Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Environmental risk assessment of glufosinate-tolerant genetically modified oilseed rape T45 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/UK/2005/25)

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

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Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Directorate for Nature Management to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The assignment includes a scientific environmental risk assessment of oilseed rape T45 (Reference EFSA/GMO/UK/2005/25) from Bayer CropScience for food and feed uses, import and processing. Oilseed rape T45 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the Norwegian Food Safety Authority related to the EFSAs public hearing in 2007 (VKM 2007a).

Food additives produced from T45 oilseed rape were notified in the EU as existing food additives within the meaning of Article 8 (1)(b) of Regulation 1829/2003, authorized under Directive 89/10/EEC (Community Register 2005). Feed materials produced from T45 were also notified as existing feed products containing, consisting of or produced from T45 according to Articles 8 and 20 of Regulation (EC) No 1829/2003 in 2003.

A notification for placing on the market of T45 according to the Directive 2001/18/EC was submitted in March 2004 (C/GB/04/M5/4), covering import and processing of T45 into food and feed. The application was further transferred into Regulation (EC) No 1829/2003 in November 2005 (EFSA/GMO/UK/2005/25). An application for renewal of authorisation for continued marketing of food additives and feed materials produced from T45 oilseed rape was submitted under Regulation (EC) No 1829/2003 in 2007 (EFSA/GMO/RX/T45). The EFSA GMO Panel performed one single comprehensive risk assessment for all intended uses of genetically modified oilseed rape T45, and issued a comprehensive scientific opinion for both applications submitted under Regulation (EC) No 1829/2003. The scientific opinion was published in January 30 2008 (EFSA 2008), and food and feed products containing or produced from oilseed rape T45 was approved by Commission Decision 26 March 2009 (Commission Decision 2009/184/EC).

The oilseed rape T45 is however currently being phased out (EU-COM 2009). The commercialisation of T45 oilseed rape seeds in third countries was stopped after the 2005 planting season and stocks of all oilseed rape T45 lines have been recalled from distribution and destroyed. The applicant commits not to commercialize the event in the future and the import will therefore be restricted to adventitious levels in oilseed rape commodity. Thus the incidence of oilseed rape T45 in the EU is expected to be limited.

The environmental risk assessment of the oilseed rape T45 is based on information provided by the notifier in the application EFSA/GMO/UK/2005/25 and EFSA/GMO/RX/T45, 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 T45 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2006, 2011a), the environmental risk assessment of GM plants (EFSA 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2006, 2011c).

The scientific risk assessment of oilseed rape T45 include molecular characterisation of the inserted DNA and expression of target proteins, comparative assessment of agronomic and phenotypic characteristics, unintended effects on plant fitness, potential for horizontal and vertical gene transfer, and evaluations of the post-market environmental plan.

In line with its mandate, VKM emphasised that assessments of sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act, shall not be carried out by the Panel on Genetically Modified Organisms.

The glufosinate ammonium-tolerant oilseed rape transformation event T45 (Unique Identfier ACS-BNØØ8-2) was developed by *Agrobacterium*-mediated transformation of protoplast from the conventional oilseed rape cultivar "AC Excel". T45 contains a synthetic version of the native *pat* gene isolated from the bacteria *Streptomyces viridochromogenes,* strain Tü 494. The inserted gene encodes the enzyme phosphinothricin acetyltransferase (PAT), which confers tolerance to the herbical active substance glufosinate ammonium. The PAT enzyme detoxifies glufosinate-ammonium by acetylation of the L-isomer into N-acetyl-L-glufosinate ammonium (NAG) which does not inhibit glutamine synthetase and therefore confers tolerance to the herbicide.

Glufosinate ammonium-tolerant oilseed rape transformation event T45 has been conventionally bred into an array of spring-type oilseed rape varieties.

Molecular characterisation

The molecular characterisation data established that only one copy of the gene cassette is integrated in the oilseed rape genomic DNA. Appropriate analysis of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analyses of junction regions demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The genetic stability of transformation event T45 was demonstrated at the genomic level over multiple generations by Southern analysis. Segregation analysis shows that event T45 is inherited as dominant, single locus trait. Phenotypic stability has been confirmed by stable tolerance to the herbicide for T45 lines and varieties derived from the event grown in Canada since 1993.

Oilseed rape transformation event T45 and the physical, chemical and functional characteristics of the proteins have previously been evaluated by The VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2007a).

Comparative assessment

Based on results from comparative analyses of data from field trials located at representative sites and environments in Canada in 1995-1997, it is concluded that oilseed rape T45 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of maturity and the herbicide tolerance conferred by the PAT protein. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of event T45 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glufosinate herbicide do not alter the phenotypic and agronomic characteristics of event T45 compared to conventional oilseed rape.

Environmental risk

According to the applicant, the event T45 has been phased out, and stocks of all oilseed rape T45 lines have been recalled from distribution and destroyed since 2005. However, since future cultivation and import of oilseed rape T45 into the EU/EEA area cannot be entirely ruled out, the environmental risk assessment consider exposure of viable seeds of T45 through accidental spillage into the environment during transportation, storage, handling, processing and use of derived products.

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

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

There is no evidence that the herbicide tolerant trait results in enhanced fitness, persistence or invasiveness of oilseed rape T45, or hybridizing wild relatives, compared to conventional oilseed rape varieties, unless the plants are exposed to herbicides with the active substance glufosinate ammonium. 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.

Accidental spillage and loss of viable seeds of T45 during transport, storage, handling in the environment and processing into derived products is, however, likely to take place over time, and the establishment of small populations of oilseed rape T45 cannot be excluded. Feral oilseed rape T45 arising from spilled seed could theoretically pollinate conventional crop plants if the escaped populations are immediately adjacent to field crops, and shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops.

However, both the occurrence of feral oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape populations to wild populations are likely to be low in an import scenario. Apart from the 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. 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.

Overall conclusion
Taking into account the expected limited import of oilseed rape T45 (EU COM 2009), the VKM GMO Panel considers that the routes of gene flow from T45 would not introduce significant numbers of transgenic plants into agricultural areas or result in any environmental consequences in Norway.

The VKM GMO Panel concludes that oilseed rape T45 is unlikely to have any adverse effect on the environment in Norway in the context of its intended usage.

Keywords

Oilseed rape, *Brassica napus* ssp. *oleifera* (DC.) Metzg., genetically modified oilseed rape T45, EFSA/GMO/2005/25, glufosinate-tolerant, *pat* gene, PAT protein, ACS-BNØØ8-2, environmental risk assessment, import, processing, Regulation (EC) No 1829/2003, Directive 2001/18/EC

Norsk sammendrag

Miljørisikovurderingen av den genmodifiserte oljerapsen T45 (EFSA/GMO/UK/2005/25) fra Bayer CropScience er utført av Faggruppen for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet (VKM). I forbindelse med forberedelse til implementering av EU-forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet bedt av Direktoratet for naturforvalting (DN) om å utarbeide endelige miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18. Oppdraget omfatter miljørisikovurdering av den genmodifiserte oljerapslinjen T45 (unik kode ACS-BNØØ8-2) fra Bayer CropScience til import og prosessering, mat og fôr i Norge. Faggruppe for genmodifiserte organismer vurderte helseaspekter knyttet til bruk av rapslinjen som næringsmiddel og fôrvare i 2007 (VKM 2007a).

Den foreløpige risikovurderingen av den genmodifiserte rapslinjen 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) og Organisation for Economic Co-operation and Development (OECD) konsensusdokument for raps (OECD 2001, 2011) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsprosess, vektor, transgene konstrukt, komparative analyser av agronomiske og fenotypiske egenskaper, 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.

Den genmodifiserte oljerapslinjen T45 har fått innsatt en genkonstruksjon med en enkeltkopi av *pat*-genet fra jordbakterien *Streptomyces viridochromogenes*. Genet koder for enzymet fosfinotricin acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium, virkestoffet i fosfinotricin-herbicider av typen Finale. Fosfinotricin er et ikke-selektivt kontaktherbicid som hemmer glutaminsyntetase. Enzymet deltar i assimilasjonen av nitrogen og katalyserer omdanning av glutamat og ammonium til aminosyren glutamin. Hemming av glutaminsyntetase fører til akkumulasjon av ammoniakk, og til celledød i planten. T45 plantene vil derfor tolerere høyere doser av sprøytemiddelet glufosinat sammenlignet med konkurrerende ugras.

Oljerapslinjen T45 inneholder ingen markørgener for antibiotikaresistens.

Molekylær karakterisering
Den transgene rapslinjen T45 har fått tilført genet *pat*. I henhold til søkers informasjon vedrørende integreringsplass og flankesekvenser til det integrerte transgenet, samt analyser vha Southern blot og sekvensering er det grunn til å tro at transgenet sitter i et lokus i genomet. Det konkluderes med at nedarvingen av *pat*-genet i rapslinjen T45 følger mønsteret for mendelsk nedarving av et enkelt, dominant lokus, og at fusjonsproteiner ikke uttrykkes i T45

Faggruppen har tidligere vurderer transformasjonsevent T45, og de fysiske, kjemiske og funksjonelle karakteriseringene av proteinet til å være tilfredsstillende (VKM 2007a). Faggruppen har ikke identifisert noen risiko knyttet til det som framkommer av den molekylærbiologiske karakteriseringen av de rekombinante innskuddene i rapslinjen.

Komparative analyser
Feltforsøk i Canada over tre vekstsesonger (1995-1997) indikerer agronomisk og fenotypisk ekvivalens mellom den transgene rapslinjen T45 og umodifisert kontroll og konvensjonelle referansesorter, med unntak av herbicidtoleranse og noe forskjell i tidlighet mellom linjene.

Miljørisiko
I henhold til søker er den genmodifiserte oljerapsen T45 trukket fra markedet og lagerpartier av T45 tilbakekalt og destruert etter vekstsesongen 2005. På bakgrunn av at framtidig dyrking av den genmodifiserte oljerapslinjen ikke kan utelukkes, er miljørisikovurderingen knyttet til mulige effekter av utilsiktet frøspredning i forbindelse med transport, lagring og prosessering av importerte partier av T45 til mat og fôr.

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

Det er ingen indikasjoner på økt risiko for spredning, overlevelse og etablering av rasplinjen T45 som naturaliserte populasjoner utenfor dyrkingsområder eller for utvikling av ugraspopulasjoner sammenlignet med ikke-transgen raps. Herbicidtoleranse er selektivt nøytralt i naturlige habitater, og kan bare betraktes å ha økt fitness hvor og når 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 i frøspill fra transport, lagring og handtering av importerte partier av rapslinje T45 kan teoretisk representere et potensiale for utkryssing og spredning av transgener til dyrkede sorter og viltvoksende populasjoner i Norge. Forekomsten av transgene 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
Tatt i betraktning den forventede begrensede import av T45 (EU COM 2009), konkluderer VKMs faggruppe for genmodifiserte organismer med at det er lite trolig at genspredning fra eventuelle ferale planter av oljeraps T45 vil resultere i etablering av transgene planter på landbruksarealer eller medføre effekter på miljø i Norge.

VKMs faggruppe for genmodifiserte organismer finner det lite trolig at utilsiktet frøspredning av rapslinjen T45 i Norge vil medføre effekter på miljøet.

Abbreviations and explanations

ALS	Acetolactate synthase, an enzyme that catalyses the first step in the synthesis of the branched-chain amino acids, valine, leucine, and isoleucine
AMPA	Aminomethylphosphonic acid, one of the primary degradation products of glyphosate
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding in maize is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred line's existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translation(s) of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards)
CTP	Chloroplast transit peptide
DAP	Days after planting
DN	Norwegian Directorate for Nature Management (Direktoratet for naturforvalting)
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue
EC	European Commission/Community
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase enzyme
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organization
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practices
Gluphosinate-ammonium	Broad-spectrum systemic herbicide
GM	Genetically modified
GMP	Genetically Modified Plant

GMO	Genetically modified organism
GMP	Genetically modified plant
H	hybrid
ha	Hectare
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
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
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.
TI	Trait integration
U.S. EPA	United States Environmental Protection Agency.
Western blot	Analytical technique used to detect specific proteins in the given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. The proteins are then transferred to a membrane where they are stained with antibodies specific to the target protein.
WHO	World Health Organisation.

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Background

Food additives produced from T45 oilseed rape (Unique Identifier ACS-BNØØ8-2) were notified in the EU as existing food additives within the meaning of Article 8 (1)(b) of Regulation 1829/2003, authorized under Directive 89/10/EEC in 1998. Feed materials produced from T45 were also notified as existing feed products containing, consisting of or produced from T45 according to Articles 8 and 20 of Regulation (EC) No 1829/2003 in 2003.

A notification for placing on the market of T45 according to the Directive 2001/18/EC was submitted in March 2004 (C/GB/04/M5/4), covering import and processing of T45 into food and feed. The application was further transferred into Regulation (EC) No 1829/2003 in November 2005 (EFSA/GMO/UK/2005/25). In addition, an application for renewal of authorisation for continued marketing of food additives and feed materials produced from T45 oilseed rape was submitted under Regulation (EC) No 1829/2003 in 2007 (EFSA/GMO/RX/T45). The EFSA GMO Panel performed one single comprehensive risk assessment for all intended uses of genetically modified oilseed rape T45 and issued a single comprehensive scientific opinion for both applications submitted under Regulation (EC) No 1829/2003. The scientific opinion was published in January 30 2008 (EFSA 2008), and food and feed products containing or produced from oilseed rape T45 was approved by Commission Decision March 26 2009 (Commission Decision 2009/184/EC).

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety has been requested by the Norwegian Directorate for Nature Management to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The assignment includes a scientific environmental risk assessment of oilseed rape event T45 for food and feed uses, import and processing. Oilseed rape T45 has previously been risk assessed by the VKM GMO Panel, commissioned by the Norwegian Food Safety Authority related to the EFSAs public hearing in 2007 (VKM 2007a).

The genetically modified oilseed rape T45 was authorized for cultivation in Canada in 1996 and the USA in 1998 (CERA 2012). Event T45 is further approved for marketing as feed and/or food in Australia, China, Japan, Korea and Mexico.

The oilseed rape T45 is however currently being phased out of the market (EU-KOM 2009). The applicant stated in the application that the sale of oilseed rape T45 derived lines by its retailers was discontinued and all T45 lines have been deregistered as of 2003 with the exception of line LL2393 that was still for sale in 2005 until exhaustion of inventory. Stocks of all other oilseed rape T45 lines has been recalled from distribution and destroyed. The applicant commits not to commercialize the event in the future and the import will therefore be restricted to adventitious levels in oilseed rape commodity. Thus the incidence of oilseed rape T45 in the EU is expected to be limited (EFSA 2008).

Terms of reference

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

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

The basis for evaluating the applicants' environmental risk assessments is embodied in the Act Relating to the Production and Use of Genetically Modified Organisms etc. (the Norwegian Gene Technology Act), Regulations relating to impact assessment pursuant to the Gene Technology Act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants (EFSA 2006, 2010, 2011a), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

The risk assessments' primary geographical focus should be Norway, and the risk assessments should include the potential environmental risks of the product related to any changes in agricultural practices. The assignment covers assessment of direct environmental impact of the intended use of pesticides with the GMO under Norwegian conditions, as well as changes to agronomy and possible long-term changes in the use of pesticides.

Assessment

1 Introduction

The glufosinate ammonium-tolerant oilseed rape transformation event T45 (Unique Identfier ACS-BNØØ8-2) was developed by *Agrobacterium*-mediated transformation of protoplast from the conventional oilseed rape cultivar "AC Excel". T45 contains a synthetic version of the native *pat* gene isolated from the bacteria *Streptomyces viridochromogenes,* strain Tü 494. The inserted gene encodes the enzyme phosphinothricin acetyltransferase (PAT), which confers tolerance to the herbical active substance glufosinate ammonium. The PAT enzyme detoxifies glufosinate-ammonium by acetylation of the L-isomer into N-acetyl-L-glufosinate ammonium (NAG) which does not inhibit glutamine synthetase and therefore confers tolerance to the herbicide. In the natural situation PAT prevents autotoxicity from bialaphos in *S. hygroscopicus* and *S. viridochromogenes*.

Transformation event T45 has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

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

The environmental risk assessment of the GM oilseed rape T45 is based on information provided by the notifier in the application EFSA/GMO/UK/2005/25, application for renewal of authorisation for continued marketing of T45 (EFSA/GMO/RX/T45), and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment is also based on a review and assessment of relevant peer-reviewed scientific literature.

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. Information related to the genetic modification

The glufosinate ammonium-tolerant oilseed rape transformation event T45 contains the *pat* gene derived from *Streptomyces viridochromogenes* (ATCC14920). The gene is a synthetic version of the native *pat* gene isolated from *S. viridochromogenes*, strain Tü 494. Since the native *pat* gene has a high G:C content, which is atypical for plants, a modified nucleotide sequence was synthesised to be adapted to the codon usage of the plant machinery. The *pat* gene encodes the enzyme phosphinothricin acetyltransferase (PAT), which confers tolerance to glufosinate-ammonium herbicides (trade names: Liberty ®, Ignite®, Finale®, Basta®). The chimeric *pat* gene construct contains the *35S* promoter of the Cauliflower Mosaic Virus, the *pat* coding sequence and the *35S* terminator of the Cauliflower Mosaic Virus. This chimeric gene that can be transferred to plants is denoted as P*35S*::*pat*::T*35S* and was inserted into the vector pPCV002. The resulting plasmid is named pHOE4/Ac(II).

2.1.1 Description of the methods used for the genetic modification

An *Agrobacterium tumefaciens* mediated transformation method was used to transform isolated *Brassica napus* protoplasts. Protoplasts were prepared from oilseed rape variety AC Excel and co-cultivated with *Agrobacterium* harbouring plasmid pHOE4/Ac(II). The proliferating calli were grown on appropriate selection medium to enrich for glufosinate ammonium-resistant tissue and later transferred to regeneration medium. Developed shoots were rooted on rooting medium followed by a transfer to a greenhouse. Acclimatised plantlets were further tested for tolerance to glufosinate ammonium, and allowed to flower and set seed.

2.1.2 Nature and source of vector used

The plasmid pHOE4/Ac(II) has essentially been derived from the vector pPCV002, and comprises the following structural elements:

- the plasmid core comprising the origin of replication from *E. coli* vector PiAN7 for replication in *E. coli* and the *ori*V and *ori*T regions from the vector RK2 for replication in *Agrobacterium tumefaciens*
- a selectable marker gene conferring resistance to streptomycin and spectinomycin (*aadA*) for propagation and selection of the plasmid in *Escherichia coli* and *Agrobacterium tumefaciens*
- an artificial T-DNA region consisting of the left border of the T-DNA from pTiAch5 and right border sequences of the T-DNA from pTiT37 and multilinker cloning sites allowing the insertion of chimeric genes between the T-DNA border repeats.

According to the applicant there are no residual T-DNA sequences present between the border repeats. The genetic elements of the vector are described in Table 1. The genetic elements transferred into the plant are described in Table 2.

Table 1. Genetic elements of the Vector pHOE4/Ac(II)

Position in vector (bp#)	Genetic element and function
0001-0060	Synthetic DNA containing the Right Border sequence from the *Agrobacterium tumefaciens* Ti plasmid pTiT37 (Depicker 1982), the Right Border goes from position 6 to 30
0061-1841	Derived from *E. coli* plasmid R538-1 containing the streptomycin/spectinomycin adenyltransferase gene (pos. 0619-1587) (Hollingshead & Vapnek 1985)
1842-2692	Derived from synthetic *E. coli* vector PiAN7 including ori ColE1 (Huang 1988)
2693-3164	Derived from *Agrobacterium tumefaciens* Ti plasmid pTiT37 (adjacent to ApaI site at pos. 60)
3165-5274	*Ori*V and *ori*T regions from E. coli RK2 plasmid (Figurski & Helinski 1979)
5275-5310	Synthetic DNA containing Left Border from *Agrobacterium tumefaciens* Ti plasmid pTiAch5 (Gielen et al. 1984), the Left Border goes from position 5282 to 5304
5311-5840	Promoter region from the Cauliflower Mosaic Virus 35S transcript from vector pDH51 (Pietrzak 1986)
5841-5868	Synthetic polylinker sequences
5869-6420	Synthetic *pat* gene from *Streptomyces viridochromogenes* (Strauch 1993)
6421-6440	Synthetic polylinker sequences
6441-6645	Terminator from the Cauliflower Mosaic Virus 35S transcript from vector pDH51 (Pietrzak 1986)
6646-6652	Synthetic polylinker sequences

Table 2. Genetic elements of Vector pHOE4/Ac(II) to be inserted into the plant genome

Symbol	Definition	Source	Size (bp)	Reference[1]	Function
LB	Left border repeat	*Agrobacterium tumefaciens*	36		*Cis*-acting element for T-DNA transfer
P-35S	Promoter	Cauliflower Mosaic Virus	530	Pietrzak (1986)	High level constitutive expression
	Polylinker sequence	Synthetic	28		Plasmid cloning site
pat	Glufosinate ammonium-tolerance *pat* gene	*Streptomyces viridochromogenes*	552	Strauch (1993)	Herbicide tolerance and selectable marker
	Polylinker sequence	Synthetic	20		Plasmid cloning site
T-35S	Terminating signal	Cauliflower Mosaic Virus	205	Pietrzak (1986)	Stop signal
	Polylinker sequence	Synthetic	7		Plasmid cloning site
RB	Right border repeat	*Agrobacterium tumefaciens*	60		*Cis*-acting element for T-DNA transfer

2.2 Information relating to the GM plant

2.2.1 Description of the trait(s) and characteristics that have been introduced or modified

The introduced trait is herbicide tolerance. The *pat* gene, when expressed, enables the production of the enzyme, Phosphinothricin-Acetyl-Transferase (PAT) that acetylates L-glufosinate ammonium and thereby confers tolerance to herbicides based upon glufosinate ammonium. This glufosinate-tolerant oilseed rape variety belongs to the species, *Brassica napus* L. and is distinguished from the recipient variety AC Excel only by tolerance to the herbicide, glufosinate, the genetic locus defined as T45, and the presence of the PAT protein. The *pat* gene was additionally used as the selectable marker.

2.2.2 Information on the sequences actually inserted or deleted

To determine the nature, number, integrity and stability of insertions in transformation event T45, PCR and Southern blot hybridisation were used. From these experiments it was concluded that T45 contains one copy only of the complete T-DNA, and that the DNA sequence of the insert is identical to the plasmid DNA sequence used for transformation. The inserted DNA has a length of 1364 bp. According to the applicant the selected transformant showed the expected phenotype of glufosinate ammonium-tolerance, confirming a functional expression of the inserted *pat* gene. The determination of inserted sequences in event T45 confirmed the presence of one copy of the *pat* gene cassette

2.2.2.1 The size and copy number of all detectable inserts, both complete and partial

According to the applicant the size and structure of the T45 insert was characterised in detail using Southern blot analysis. DNA was extracted from leaves from four-week-old greenhouse-grown rapeseed plants for both T45 and the non-transgenic counterpart AC Excel. Plant DNA was extracted and digested with restriction enzymes and separated according to size by agarose gel-electrophoresis. The schematic representation of the DNA fragment comprised between the right and left border repeat of pHOE4/Ac(II), the insert in oilseed rape event T45 and the probes used, are outlined in Figure 1. The restriction enzymes used with the three different probes are marked and the cross-hybridising bands with the respective probes are highlighted.

The Southern blot hybridisation results obtained with oilseed rape event T45 showed that the transferred DNA in the plant genome corresponds to the DNA configuration as designed in the pHOE4/Ac(II) plasmid vector. The verified region spans from bp 5305 until bp 6647 (the *Eco*RI fragment carrying the *pat* gene cassette) in the pHOE4/Ac(II) plasmid. One copy only of the gene cassette is integrated in the oilseed rape event T45, vector sequences are not integrated. PCR analyses have shown that the integrated DNA is limited to the DNA between the T-DNA border repeats. The insert has also been sequenced and the presence of one single copy of the T-DNA confirmed.

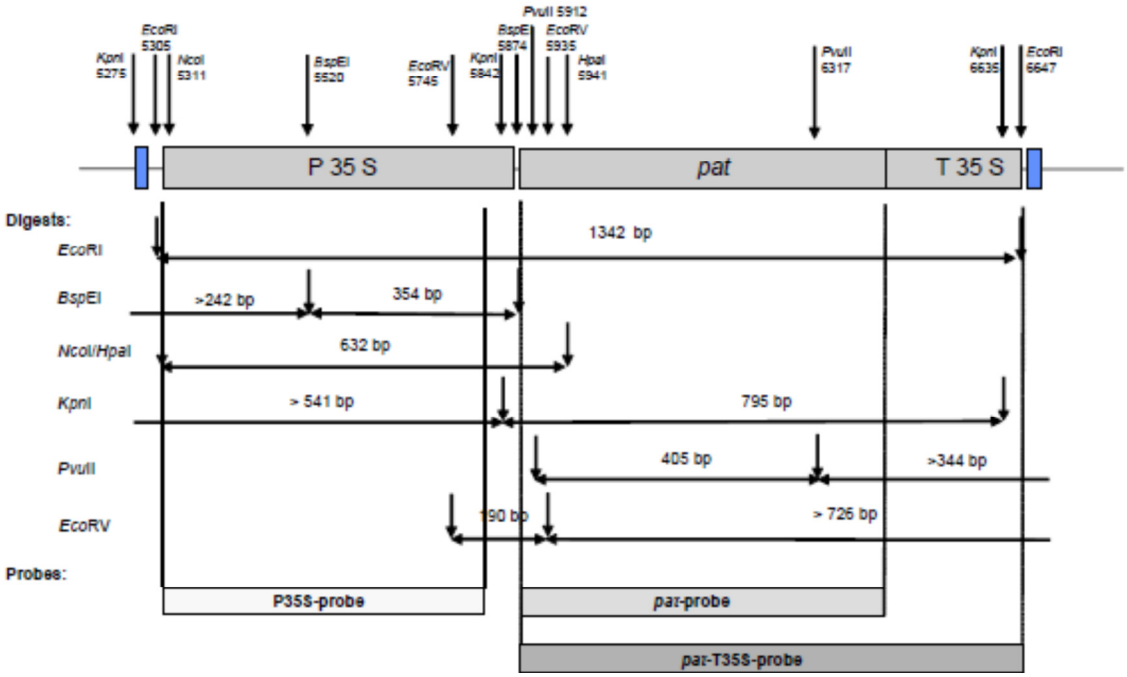

Figure 1. Schematic drawing of the T-DNA region in oilseed rape event T45

2.2.2.3 The organization of the inserted genetic material at the insertion site and methods used for characterization

According to the applicant the oilseed rape event T45 specific integration sequences including the Right Border flanking sequences (911 bp) and the Left Border flanking sequences (994 bp) were determined. The specific integration sequences were determined using the thermal asymmetric interlaced PCR method (TAIL-PCR). This method entails consecutive reactions with nested sequence-specific primers and a shorter arbitrary degenerate primer (some of its positions have several possible bases). The method allows the isolation of DNA segments adjacent to known sequences. The analysis demonstrated that the characterised RB and LB flanking sequences are of oilseed rape plant origin.

Determination of the wild type target locus sequence (pre-insertion sequence) was performed using DNA isolated from wild type oilseed rape DNA. A flanking DNA specific primer upstream of the T-DNA insert was used together with a flanking DNA specific primer downstream of the T-DNA insert to amplify the target site. A fragment of about 1900 bp was amplified and sequenced. The obtained sequence was aligned with the 5-prime and 3-prime flanking sequences of T45. The alignment showed that the sequences flanking the T45 locus are identical to the corresponding sequences in the pre-insertion locus. A fragment of 48 bp is present at the wild type target locus but missing in the transgene locus. These 48 bp were deleted (target site deletion) upon integration of the T-DNA in the genomic DNA. At the 5-prime junction 9 bp are inserted that are not present in the pre-insertion locus. These 9 base pairs are a duplication of 3-prime flanking sequences. Two bp are inserted at the 3-prime junction.

2.2.2.4 In the case of deletion(s), size and function of the deleted region(s)

According to the applicant it is not likely that the 48 bp that were deleted upon integration belong to a coding sequence as indicated by homology searches of the flanking sequences.

2.2.2.5 Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria or maintained in a non-integrated form) and methods for its determination

The insert in T45 is integrated in the chromosomal genome of *B. napus*. In Southern blot analysis with non-digested T45 DNA, a high molecular weight fragment was observed with every probe used, indicating that the transgenic sequences are integrated into the oilseed rape genomic DNA. Normal Mendelian segregation was observed in further generations. The transgene inherits as a single dominant trait, which indicates chromosomal integration of the insert.

2.3 Information on the expression of the insert

2.3.1 Part of the plant where the insert is expressed

2.3.2 The range and mean values for the levels of PAT protein

RNA expression levels of the *pat* gene were found in the following order: leaf, stem > root, but not detected in mature seeds. However, the PAT protein was demonstrated in seeds at 0.0027% (w/w) of extracted total protein, i.e. 930 ng/g dry weight (Table 4). According to the applicant, there was no evidence of a decreased expression of the PAT enzyme over time as indicated by acceptable tolerance of the plants treated with glufosinate ammonium (GA, trade name Liberty®) under a wide variety of field conditions and crop growth stages during the development and commercial sale of T45 in North America over a period of 6 years.

The PAT protein has been quantified in both green leaf tissue and in seeds from filed trials at four locations in Canada in 1996 (Technical Dossier: Deschamps 1997). The protein is detectable at relatively low levels in both seeds and green leaf tissue (100 - 1000 ng/g total extractable protein (TEP)).

After sampling in fields, crude protein extracts of oilseed rape seeds and leaves were analysed for the presence of the PAT protein by an enzyme activity assay using a specially designed ELISA test kit (Technical Dossier: Beriaut 1999). The results are summarised in Table 3 and 4.

2.3.3 Expression of potential fusion proteins

Bioinformatic analysis performed on the gene insertion site, the flanking regions and the plant DNA junctions revealed 36 putative Open Reading Frame sequences (ORFs, minimal size of 3 amino acids), of which four were newly created due to the insertion event. Analysis of the first ATG codon context sequence, promoter and 3'untranslated sequences showed that none of these ORFs can be considered as transcriptionally and/or transnationally active. No significant sequence similarities with known toxins or allergens were found. There were also no indications that the T-DNA is integrated in a coding region of the wild-type oilseed rape genome, which is supported by observations from several field trials showing no alteration in the plant's phenotype.

Table 3. Summary of PAT protein levels in tissues of oilseed rape T45 from the field trials in Canada in 1996 (Deschamps 1997).

Tissue	Location	Herbicide treatment	ng PAT/g sample	
			mean	SD
Leaf	Yorkton, SK	untreated	875	45
	Yorkton, SK	treated	959	92
	High Bluff, MD	untreated	522	65
	High Bluff, MD	treated	588	61
	Rosthern, SK	untreated	745	242
	Rosthern, SK	untreated	911	334
	Innisfail, AB	treated	791	259
	Innisfail, AB	untreated	768	208
Mean			**769.88**	
Grain	Yorkton, SK	untreated	562	120
	Yorkton, SK	treated	468	104
	High Bluff, MD	untreated	717	123
	High Bluff, MD	treated	735	84
	Rosthern, SK	untreated	681	105
	Rosthern, SK	treated	574	105
	Innisfail, AB	untreated	690	96
	Innisfail, AB	treated	574	113
Mean			**625.13**	

Table 4. PAT content in T45 seed from plants treated with Liberty herbicide (496.83 g a.i./ha) and non-transgenic, non-treated seed

Product	Mg TEP/g fw Mean ± SD	ng PAT/g fw Mean ± SD	Moisture %	Ng PAT/g dm Mean ± SD	% PAT/ TEP
Non-transgenic seed	25.4 ± 4.4	ND			
T45 seed, herbicide treated	32.3 ± 2.2	877 ± 51	5.7	930	0.0027

The TEP and PAT values are expressed as mean and standard deviation of four assay results from duplicate extracts; two assay results for each extract.
ND: Not detectable

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

2.4.1 Genetic stability of the insert in T45

To demonstrate the stability of oilseed rape T45 event over multiple generations, Southern blot analysis, using the 1342 bp *Eco*RI fragment carrying the *pat* gene cassette, was performed. Three different generations were used: F5 (also referred to as R1:F5, the fourth selfed generation of the cross R1 x AC Excel, where R1 plants are the first generation of tolerant, seed derived progeny from the first self-pollination of R0 plants, the primary regenerates); F7 (also referred to as R1:F7, the sixth selfed generation of the cross R1 x AC Excel) and R2 (the first generation of selfed R1 plants). DNA from T45 plants was digested with *Hpa*I. This restriction enzyme has only one restriction recognition site in the transforming T-DNA. All three generation were found to have identical integration fragments demonstrating the stability of the oilseed rape T45 event at the genomic level over multiple generations. A schematic drawing of the strategy is presented in Figure 2.

2.4.2 Phenotypic stability of the GM plant

Fifteen R1 plants were grown in pots in a growth room and sprayed with glufosinate ammonium (equivalent of 400g a.i./ha) at the four leaf stage. R1 transgenic plants were expected to be either homozygous or hemizygous for the inserted transgene. Five to 10 glufosinate ammonium-tolerant R1 plants were crossed with the susceptible non-transgenic cultivar. It was hypothesized that R1:F1 families would segregate tolerant and susceptible plants or contain tolerant plants only. The former condition would indicate hemizygosity and the latter homozygosity for the transgene. A single plant from each non-segregating R1:F1 family was selfed and reciprocally crossed to its susceptible parent cultivar to obtain both, R1:F2 and R1:BCF1 progeny. Glufosinate ammonium was applied and the number of tolerant and susceptible plants for each selfed and crossed progeny recorded (Table 5). Results were tested using chi-square for goodness-of-fit to expected Mendelian ratios. The results show that the T45 insert inherits as a dominant, single locus trait. In addition it is argued by the applicant that phenotypic stability has been demonstrated, as T45 derived lines and varieties grown in Canada since 1993 have displayed consistent tolerance to the herbicide.

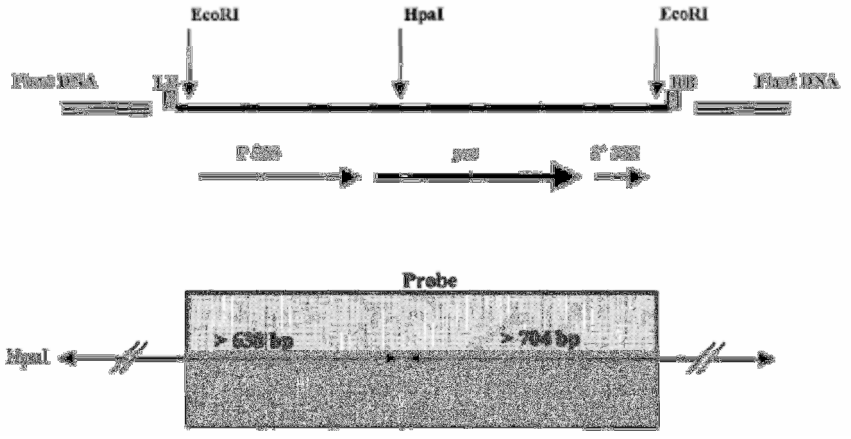

Figure 2. Schematic drawing of the hybridisation strategy

Table 5. Segregation analysis of progeny from oilseed rape T45

Parents and zygosity for the *pat* locus	Generation	Ratio R:S	Observed		Expected		χ^2 test	
			R	S	R	S	3:1	1:1
R_1:F_1 (hemizygous) self [(pat/-)x(pat/-)]	R_1:F_2	3:1	54	18	54	18	ns	*
R_1:F_1 (hemizygous)x AC Excel [(pat/-)x(-/-)]	R_1:BCF_1	1:1	35	37	36	36	*	ns

S= susceptible,
R= resistant,
*= significantly different from the tested ratio (P = 0.05),
ns= not significantly different from the tested ratio (P = 0.05).

2.5 Conclusion

The molecular characterisation data established that only one copy of the gene cassette is integrated in the oilseed rape genomic DNA. Appropriate analysis of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analyses of junction regions demonstrated the absence of any potential new ORFs coding for known toxins or allergens.

The genetic stability of transformation event T45 was demonstrated at the genomic level over multiple generations by Southern analysis. Segregation analysis shows that event T45 is inherited as dominant, single locus trait. Phenotypic stability has been confirmed by stable tolerance to the herbicide for T45 lines and varieties derived from the event grown in Canada since 1993.

Oilseed rape event T45 and the physical, chemical and functional characteristics of the proteins have previously been evaluated by The VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2007a).

3 Production, import and use of oilseed rape

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

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

In Norway, the acreage used for cultivation of oilseed rape has varied significantly during the past 15 years (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 Norwegian imports of rapeseed oil in 2007 amounted to 1,136,431 tonnes (SLF 2008). With the exception of 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.

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

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

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

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

Due to the high performance requirements for livestock production, farmers are demanding ever more protein-rich feed types. This has led to a large increase in the import and use of protein ingredients such as rapeseed meal (SLF 2011). According to statistics from the Norwegian Agricultural Authority, 91 100 tonnes of processed rapeseed (pellets/meal) were imported in 2010 as a raw protein product for use in the Norwegian feed concentrate production (SLF 2011). Similarly, over 8 000 tonnes of oilseeds were imported for 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 2010, 11 500 tonnes of Norwegian-produced oilseeds were used for the production of feed (SLF 2011).

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

4 Comparative assessment

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

Experimental design
The application EFSA/GMO/UK/2005/25 for food and feed uses, import and processing of oilseed rape T45 within the European Union, presented compositional data from seed and forage material collected in field trials in Canada in 1995, 1996 and 1997 (Technical Dossier: Weston 2005). The primary objective of the so called "Co-test" was to gather data indicating the relative performance of the candidate and check cultivars under condition reflecting local commercial production practices. The first year trials are private data trials, while the second and third year trials are public data trials. According to CERA (2012) the same oilseed rape line (HCN-28, derived from T45) has been field tested in the United States (1996, 1997), and in Chile, Japan, Great Britain, and Australia. Data from these field trials are, however, not attached to the application EFSA/GMO/UK/2005/25.

The Canadian field trials compared the composition of event T45 with a conventional counterpart having a comparable genetic background. The comparator included in the field trials was the commercial oilseed rape variety "AC Excel", which was used as the recipient for the DNA insertion to establish transformation event T45. The applicant stated that although T45 has been transformed in an AC Excel background and is the correct counterpart the line HCN-28 has been derived from selections from that population, consequently some differences can be anticipated as there is no exact isogenic counterpart to this material for comparison. EFSA Regulation (EC) No 1829/2003 defines a conventional counterpart as „a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use (Art. 2.12). In line with this legal requirement the EFSA GMO Panel provides details on the criteria for the selection of appropriate comparators, under different scenarios, in the EFSA Guidance for the Selection of comparators for the risk assessment of GM plants (EFSA 2011b).

The conventional commercial reference varieties "Cyclone" and "Legend" were also included in the comparative assessments to provide a range of comparative values that are representative of existing conventional reference varieties for each measured phenotypic or agronomic characteristic. The commercial varieties used in these studies were selected to represent a range of genetic backgrounds and phenotypic characteristics and have been grown in the oilseed rape production regions. According to the applicant, they also reflect a range of data on natural variability within commercial oilseed rape varieties and therefore can provide context for interpreting experimental results.

The field trials were performed at nine locations with a long growth season and 13 locations in mid-season growing area. In addition, short season trials were performed at seven locations. All the experimental locations were representative of oilseed rape cultivation areas in Canada. The variety trials were accomplished prior to registration and were performed according to the VWCC/RCC guidelines.

At each trial site, oilseed rape T45, the conventional counterpart and the reference varieties were planted following a randomized complete block design with minimum 3 replicates per site (4 replicated recommended). At all sites, each plot was planted with four passes of a seed drill (5 metres long). Plots were separated by a 9-10 m conventional rape seed buffer in order to limit edge effects.

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

Statistical analysis
The variety trials were accomplished in 1995, 1996 and 1997 prior to registration and were performed according to the WCC/RCC guidelines using standard statistical analyses. The data set collected in the 1996 field season, as it represent the largest data set collected in the 1996 field season, has been pooled and subjected to ANOVA analysis and a series of descriptive statistics are presented to further describe the mean values.

4.2 Agronomic traits and GM phenotype

During field trials conducted over three seasons and different locations, observations on plant height, lodging resistance at maturity, plant development and days to maturity, disease (blackleg) resistance and yield were collected. The evaluation scale used for blackleg resistance is a visual rating ranging from 0 to 5, where 0 is no infection, 3 = approximately ¾ of the stem circumference lesioned and 5 stem completely severed/plant dead. A correspondingly visual rating scale was used for the evaluation of lodging resistance.

4.2.1 Agronomic and phenotypic results

For all parameters evaluated in the Canadian field trials in the second growth season, there was no significant difference observed ($p<0.05$) (Table 6). The data presented supports the agronomic equivalence of the event T45 with its non-transgenic counterpart.

Table 6. Phenotypic and agronomic characteristics of oilseed rape T45 (Canadian field trials 1996)

Characteristics	AC Excel Mean (SD)	T45 Mean (SD)	P-value	Significance
Plant height (cm)	117.7 (15.9)	121.9 (15.3)	0.433	ns
Blackleg resistance (0.5)	1.67 (0.536)	1.25 (0.326)	0.286	ns
Lodging (0.5)	2.0 (0.64)	2.1 (0.53)	0.896	ns
Days to maturity (days)	96.8 (12.5)	98.2 (13.2)	0.835	ns
Yield (kg/ha)	2480 (824)	2714 (936)	0.429	ns

Mean values for the different agronomic data from the growth seasons 1995-1997 are summarised in tables 7-11. According to the applicant, no major differences in plant morphology, growth and plant development were observed. Event T45 (HCN-28) is in average taller than the conventional comparator and the reference varieties (Table 8). Lodging resistance varies according to season and field site, but is not essentially different from the other oilseed rape cultivars included in the field study. Oilseed rape T45 tends to flower and subsequent mature later than the comparators (Table 10).

Stress adaption was evaluated, including resistance to major oilseed pests as blackleg (*Leptospaeria maculans/Phoma lingam*) and determined to fall within the ranges currently displayed by commercial varieties (Table 11).

Table 7. T45 (HCN-28) yield data from variety trials in Canada in 1995, 1996 and 1997

Cultivar	Yield (kg/ha) Long season (irrigated)			Yield (kg/ha) Mid-season			Yield (kg/ha) Short season	
	1995 5 sites	1996 7 sites	1997 8 sites	1995 6 sites	1996 8 sites	1997 7 sites	1995 5 sites	1996 3 sites
Cyclone	2656	2833	2407	1900	2672	2224	3199	2721
Legend	2350	2660	2156	2077	2320	2042	2898	2428
AC Excel	2171	2685	2185	1856	2383	2088	3001	2710
T45 (HCN-28)	2635	2893	2260	2066	2568	2181	2734	2683
Check mean	2392	2627	2249	1944	2458	2127	3033	2620

Table 8. T45 (HCN-28) plant height data from variety trials in Canada in 1995 and 1996

Cultivar	Plant height (cm) Long season (irrigated)		Plant height (cm) Mid-season		Plant height (cm) Short season	
	1995 5 sites	1996 8 sites	1995 5 sites	1996 6 sites	1995 5 sites	1996 3 sites
Cyclone	135	124	105	112	83	101
Legend	129	127	101	112	73	104
AC Excel	136	117	107	106	81	97
T45 (HCN-28)	146	130	108	118	88	108
Check mean	134	123	104	110	79	108

Table 9. T45 (HCN-28) lodging resistance data from variety trials in Canada in 1995 and 1996

Cultivar	Lodging resistance (0-5)[1] Long season (irrigated)		Lodging resistance (0-5) Mid-season		Plant height (cm) Short season	
	1995 5 sites	1996 7 sites	1995 2 sites	1996 2 sites	1995 5 sites	1996 1 site
Cyclone	0.8	2.5	2.4	1.8	2.0	2.3
Legend	1.1	2.7	1.9	1.9	2.5	2.3
AC Excel	1.1	2.7	1.9	2.7	2.4	2.3
T45 (HCN-28)	1.5	2.5	2.1	2.0	2.5	2.3
Check mean	1.0	2.6	2.1	2.1	2.3	2.3

[1] 0=no lodging, 5=flat

Table 10. T45 (HCN-28) maturity data from variety trials in Canada in 1995 and 1996

Cultivar	Days to maturity Long season (irrigated)		Days to maturity Mid-season		Days to maturity Short season	
	1995 5 sites	1996 8 sites	1995 3 sites	1996 5 sites	1995 5 sites	1996 2 sites
Cyclone	91	89	104	92	90	110
Legend	89	90	103	91	87	111
AC Excel	90	88	103	91	91	110
T45 (HCN-28)	92	91	105	92	92	113
Check mean	90	89	103	91	89	110

Table 11. T45 (HCN-28) blackleg resistance data from variety trials in Canada in 1995 and 1996

Cultivar	Blackleg resistance (0-5)[1] 1995	Blackleg resistance (0-5) 1996
Westar	Nd	3.3
Apollo	Nd	2.2
Cyclone	0.7	1.0
AC Excel	1.3	1.6
Legend	1.2	1.3
Legacy	Nd	1.6
T45 (HCN-28)	1.1	1.3
Check mean	1.1	1.3

[1] 0=no symptoms, 5= severe symptoms or dead
Nd= not determined

4.2.2 Invasive potential and competing ability

Oilseed rape T45 (line HCN-28) and three commercial oilseed varieties (AC Excel, Legend and Cyclone) were further investigated in a replacement series experiment under field conditions at three locations in western Canada (Technical Dossier: Belyk & McDonald 1995). The plots were rated for vegetative growth (above-ground biomass) prior to bolting and used to evaluate the competitive ability and aggressively of HCN-28 with its non-transgenic counterparts. The transgenic line HVN-28 was grown in monoculture and in mixed populations with one of three standard commercially rapeseed cultivars. Each series consisted of two monocultures and the three mixtures 25/75, 50/50 and 72/25 planting ratios. All plots were treated with 800 g ai/ha glufosinate ammonium (Liberty^{TM}) when the plants had approximately 5 leafs and had not yet bolted.

Results from evaluations indicate no qualitative differences between T45 and the conventional counterpart with respect to invasive potential and competing ability under agronomic conditions. Values of aggressively were determined to provide a measurement of competitiveness between HCN-28 and the Legend, AC Excel or Cyclone varieties. An analysis of variance across all locations indicated no significant differences between the transgenic line and the commercially varieties tested, and absence of increased weediness potential in T45. According to the applicant, no further observations of pleiotropic or epistatic effects due to the insertion of the *pat* gene have been made.

4.3 Conclusion

Based on results from comparative analyses of data from field trials located at representative sites and environments in Canada in 1995-1997, it is concluded that oilseed rape T45 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of the herbicide tolerance conferred by the PAT protein and maturity. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of event T45 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glufosinate herbicide do not alter the phenotypic and agronomic characteristics of event T45 compared to conventional oilseed rape.

5 Environmental risk assessment

The application EFSA/GMO/UK/2005/25 under Regulation (EC) No 1829/2003 is for the authorisation of genetically modified oilseed rape T45 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.

According to the applicant, the event T45 has been phased out of the market, and stocks of all oilseed rape T45 lines have been recalled from distribution and destroyed since 2005. However, since future cultivation and import of oilseed rape T45 into the EU/EEA area cannot be entirely ruled out, the environmental risk assessment consider exposure of viable seeds of T45 through accidental spillage into the environment during transportation, storage, handling, processing and use of derived products.

5.1 Reproduction biology of oilseed rape

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

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

The pollen from oilseed rape can be transferred from plant to plant through physical contact between flowers of neighbouring plants and/or by wind and pollinating insects (Eastham & Sweet 2002; OECD 2011). The relative importance of wind versus insect pollination is unclear and probably varies with location and weather. The rape pollen grains have features that are typical of insect pollination being relatively large (32-33 µm), heavy and sticky (OECD 2011; Treu & Emberlin 2000). The flowers of oilseed rape produce nectar with relatively high concentrations of sugars and have a colour and structure which makes them attractive to insects, particularly bees. Honeybees (*Apis melifera*) are an important insect pollinator of oilseed rape in Scandinavia, followed by bumblebees (*Bombus* sp.), and Brachycera (Tolstrup et al. 2003; VKM 2007b). 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 an 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, recently dormant oilseed rape seed has been found in non-till systems, indicating that seed can fall dormant on the soil surface, and need not to be buried in the dark (Gruber et al. 2010).

5.2 Unintended effects on plant fitness due to the genetic modification

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

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

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

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

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

Nishizawa et al. 2009; Schafer et al. 2011). If habitats are disturbed on a regular basis by anthropogenic activities, such as mowing, herbicide applications or soil disturbance, or natural occurrences, such as flooding, then feral populations can persist for longer periods (Claessen et al. 2005a; Garnier et al. 2006). The underlying ecological processes associated with the establishment and persistence of such populations has, however, rarely been investigated (Pivard et al. 2008a).

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

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

Results from the European research project SIGMEA show that there is little establishment of naturalised populations of oilseed rape plants outside of agricultural areas in northern Europe (Tolstrup et al. 2007). The project, which included studies of feral oilseed rape plants on roadsides, field margins, and waste lands in Denmark, Germany, UK and France (covering a total of 1,500 hectares and 16 years of observation), documented generally low frequencies of naturalised populations (on average, one population (1-10 plants) per km^2). In the Danish study, 12 flowering plants/km^2 were recorded over two growing seasons. In France, the study was localised to areas with extensive oilseed rape cultivation, and showed significantly higher frequencies of escaped oilseed rape populations (15 populations/km^2) (Lecomte et al. 2007).

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

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

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

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

There is no evidence 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 GMHT oilseed rape is neither more likely to survive nor to be more persistent or invasive compared with its non-GM comparator. The herbicide tolerance trait can only be considered to be a selective advantage when the GM plants are sprayed with glyphosate- or glufosinate-ammonium containing herbicides. In addition, the ability of invasion of ruderal habitats also appears to be limited by areas for seed germination and competition from other vegetation.

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

Field trials with the oilseed rape cultivar T45 in representative areas for oilseed rape cultivation in Canada have shown equivalence between the transgenic line and the corresponding, unmodified control with respect to agronomic and phenotypic characteristics. With the exception of tolerance to glufosinate ammonium, according to the applicant no evidence of significant differences with respect to the characteristics associated with reproduction and vegetative growth have been demonstrated in these field studies, between the oilseed rape cultivar and conventional varieties with equivalent genetic backgrounds. Investigations of interactions between the oilseed rape cultivar T45 and biotic and abiotic factors, as well as studies of seed dormancy, seed germination, morphology, and pollen vitality, indicate no unintended effects of the introduced characteristics on the phenotypic characteristics of T45.

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.

5.3 Potential for gene transfer

A prerequisite for any gene transfer is availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via seed spillage followed by cross-pollination. Considering the scope of the application and the physical characteristics of oilseed rape seeds, possible pathways of dispersal are from: (1) occasional oilseed rape plants originating from indirect exposure through manure and faeces from gastrointestinal tracts of animals fed on GM oilseed raps; (2) accidental spillage of viable T45 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 T45.

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

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

5.3.1 Plant-to-microorganism gene transfer

Experimental studies have shown that gene transfer from transgenic plants to bacteria rarely occurs under natural conditions and that such transfer depends on the presence of DNA sequence similarity between the DNA of the transgenic plant and the DNA of the bacterial recipient (Nielsen et al. 2000; de Vries & Wackernagel 2002, reviewed in EFSA 2004, 2009; 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 T45 to unrelated species such as bacteria.

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

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

The origin and properties of the inserted gene does not suggest a novel directional positive selection of the plant transgenes in T45 in bacterial recipients.

In conclusion, the VKM GMO Panel consider it is unlikely that genes from T45 will transfer and established in the genome of microorganisms in the environment or in the intestinal tract of humans or animals

5.3.2 Plant-to-plant gene flow

The potential for cross-pollination between oilseed rape cultivar T45 and conventionally bred oilseed rape varieties, other cultivated *Brassica* species, related species, or overspill oilseed rape plants occurring as weeds in agricultural areas or in natural or semi-natural habitats, depends on the extent of accidental seed dispersal and the establishment of overspill plants in association with transport, storage, handling, and further processing. Several studies investigating gene exchange with related wild plants or other cultivated varieties or species of agricultural plants have been published. However, these studies are mostly related to the cultivation of oilseed rape, either in field trials or commercial fields for cultivation. Little data have been published that can elucidate the potential for spread and integration of transgenes from dispersed escaped plant populations or from populations under different environmental conditions.

5.3.2.1 Potential for cross-pollination with cultivated oilseed rape varieties

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

The majority of out-crossing occurs within the first 100 metres. Data from over 100 field trials with spring and winter oilseed rape in the British FSE-Project ('Farm Scale Evaluation') have been used to predict unintended introduction of transgenes into harvested seeds as a function of, among other factors, isolation distance and field size (length/width) (Weekes et al. 2005; NIAB 2006). The results from this study showed that when plants were used that contained two transgene copies, less than 0.3 % introduction was registered in conventional crop fields at distances of 35 metres, given a field depth of 200 metres. In those cases where pollen competition from the donor field was reduced by halving the width of the field, the introduction increased by 0.6 % and 0.8 % for winter and spring oilseed rape, respectively. For comparison, a less than 0.4 % introduction was found when using hemizygotic plants in field widths of 100 metres.

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

Feral oilseed rape T45 arising from spilled seed could theoretically pollinate conventional crop plants if feral populations are immediately adjacent to field crops, and shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops. However, the frequency of such events is

likely to be extremely low. Squire et al. (2011) and Devos et al. (2012) concluded that this route of gene flow would not introduce significant numbers of transgenic plants into agricultural areas or result in any environmental consequences.

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

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

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

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

Interspecific and intergeneric sexual crossing attempts, degree of success and potential for gene introgression with different species in the cruciferous family are presented in Table 12 (OECD 2011). A summary of some of these studies are presented in the following paragraphs and discussed in more details in the Appendix 2.

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

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

B. napus x Raphanus raphanistrum *R. raphanistrum x B. napus*	Y	F						al. 1994; Lefol et al. 1997; Rieger et al. 1999; Chevré et al. 1997a, 1998
B. napus x R. sativus *R. sativus x B. napus*	Y NR	NR	0					Gupta 1997; Ammitzbøll & Jørgensen 2006
R. napus x Eruca sativa *E. sativa x B. napus*	Y NR	NR NR				L	VL	Birjal & Sharma 1996b
B. napus x Erucastrum gallicum *E. gallicum x B. napus*	Y F	F NR	0.1 0	Y	Y	VL VL	VL VL	Lefol et al. 1997; Warwick et al. 2003
B. napus x Sinapis alba *S. alba x B. napus*	Y F	NR NR	Y			VL EL	VL EL	Chevré et al. 1994; Brown et al. 1997
B. napus x S. arvensis *S. arvensis x B. napus*	Y Y	F F	0.18 F	F		L EL	VL EL	Bing et al. 1991; Moyes et al. 2002; Sweet et al. 2007; Lefol et el. 1996b.

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

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

Interspecific hybrids between *B. napus* and *B. rapa* are mostly triploid, with reduced pollen fertility, and hence low ability to pollinate and form backcrosses with *B. napus* (Jørgensen & Andersen 1994; Norris et al. 2004; Warwick et al. 2003). The survival rate of hybrid seedlings is also low (<2 % survival) (Scott & Wilkinson 1998), reducing the rate of introgression (Jørgensen et al. 1996). Introgression of HR transgenes from *B. napus* to *B. rapa* has occurred in Europe (Jørgensen 1999; Hansen et al. 2001; Norris & Sweet 2002). Extensive introgression has e.g. been reported from a mixed population of *B. napus* and *B. rapa* in organically farmed fields in Denmark, 11 years after conversion (Hansen et al. 2001). Of 102 plants analysed, only one individual was a first generation hybrid (F_1-hybrid), while almost half of the plants had specific genetic markers from both *B. napus* and *B. rapa*. An UK study of naturally occurring wild turnip in GM oilseed rape also showed a high incidence of hybridization between these species (Norris et al. 2004)

The first report that documents the persistence and stable incorporation of transgenes from herbicide-resistant oilseed rape into *B. rapa* ssp. *campestris* in commercial cultivation fields was published in 2008 by Warwick et al. (Warwick et al. 2008). This study confirmed the persistence of a glyphosate tolerance trait over a period of 6 years in a population of *B. rapa* in the absence of selective pressure in the form of glyphosate treatment and in spite of fitness costs associated with hybridisation. This was demonstrated in both F_1-generations and backcrossed generations of the hybrid. Elling et al. (2009) measured the extent of hybridisation between autotetraploid *B. rapa* varieties (female) and *B. napus* (pollen donor) under experimental field conditions and found that the hybridisation with tetraploid *B. rapa* seemed to be more likely than with diploid *B. rapa.* The authors reported higher pollen fertility in these hybrids than thos formed with diploid *B. rapa* and suggested that introgression frequencies from *B. napus* to *B. rapa* would be higher in tetraploid *B. rapa*. They also reported the presence of some feral tetraploid *B.rapa* populations in Germany, but did not report on interspecific hybrids or backcrosses in these populations. Surveys conducted in Japan did not detect transgenes in seed collected from wild relatives of *B. napus* (*B. rapa* and *B. juncea*) sampled at ports, and along roadsides and riverbanks (Saij et al. 2005).

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

Mustard greens/brown mustard (*B. juncea* (L.) Czern.)
Hybrids have been produced by controlled crossings between oilseed rape and mustard greens (Mikkelsen & Jørgensen 1997). It is also known that the hybrids can form spontaneously under natural field conditions (Frello et al. 1995; Jørgensen et al. 1996; Liu et al. 2010). In a Danish study, Jørgensen et al. (1996) reported a 3 % hybridization frequency from crossings with *B. napus* as a pollinator. Equivalent results have been reported from Canada (Bing et al. 1991; Eastham & Sweet 2002). Species hybridization can occur in both directions, but is most successful with *B. napus* as the

pollen donor. The F_1-hybrid has low fertility (0 – 28 %), but expression of transgenes has been observed in the first generation after backcrossing to *B. juncea* (Jørgensen 1999).

Mustard greens is an annual, introduced plant in Norway, located on waste ground in Southern Norway (Lid & Lid 2005). The species is now considered as established in Norway.

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

Hoary mustard (*B. adpressa* Boiss.)
B. adpressa can produce F_1 hybrids with *B. napus* (Lefol et al. 1996). The introgression of *B. napus* genes into *B. adpressa* is, however, not likely to be a significant phenomenon because the hybrids have decreased fitness, reduced seed production, no viable seed and irregular chromosome numbers of the plants in each backcross generation with abortion of *B. napus* chromosomes frequently occurring (Darmency & Fleury 2000).

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

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

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

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

Annual wall rocket (*Diplotaxis muralis*)*,* **perennial wall rocket** (*D. tenuifolia* (L.) DC)
Hand crosses have been made in enclosed environments between *B. napus* and *Diplotaxis muralis* and *D. tenuifolia.* No field interspecific or intergeneric hybrids have been reported between and these species (ref. OECD 2011).

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

5.4 Potential interactions of the GM plant with target organisms

Interactions of oilseed rape T45 with target organisms are not considered an issue by the VKM Panel on Genetically Modified Organisms, as there are no target organisms.

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

The scope of this application covers import and processing, and all uses as any other oilseed rape excluding cultivation. No deliberate release of viable plant material in the EU/EEA is expected and interactions of T45 with the biotic environment will be very limited. Some accidental spillage of seed from T45 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 T45 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 T45 is negligible.

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

Cultivation of oilseed rape T45 in the EU is not included in the scope of the application EFSA/GMO/UK/2005/25. An assessment of the impacts of altered cultivation, management and harvesting techniques of T45 is therefore not relevant given the scope of this application.

5.7 Potential interactions with the abiotic environment and biogeochemical cycles

The scope of the application covers import, processing, and food and feed use of oiseed rape T45, and no deliberate release of viable plant material is expected in the EU/EEA and interactions of T45 with the biotic environment will be very limited. The limited routes of exposure of soil micro-organisms to T45 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 T45. 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 T45 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 T45 in the EU it is not likely to adversely impact soil

micro-organisms that perform ecological functions in-field or in non-agricultural habitats, and therefore poses negligible environmental risk.

6 Post-Market Environmental Monitoring Plan

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

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

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

6.1 Case-specific GM plant monitoring

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

The scope of the application EFSA/GMO/UK/2005/25 is the authorisation of T45 for import, processing and the use of food and feed produced from T45 in the EU under Regulation (EC) No. 1829/2003. The commercialisation of T45 oilseed rape seeds in third countries was however stopped after the 2005 planting season and this event is currently being phased out. Therefore event T45 will only be present in low level in the import commodities.

The environmental risk assessment, conducted by the applicant, support a conclusion that the import, processing and all uses as any other oilseed rape, but excluding the cultivation of T45 in the EU, represents negligible risk to human and animal health and the environment, and poses no greater risk than the import and processing of conventional oilseed rape. The applicant has therefore considered that there is no need for case-specific monitoring.

Due to the limited exposure, and this only at import facilities or processing plants, it is unlikely that a possible spill of oilseed rape T45 will have any influence on human or animal health or the environment.

6.2 General surveillance for unanticipated adverse effects

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

In the context of the intended uses of T45, exposure to the environment will be limited to unintended release of rape seed, which could occur e.g via losses during loading/unloading of viable commodity including T45 destined for processing into animal feed or human food products.

The applicant proposed to conduct general surveillance for oilseed rape T45 throughout the period of validity of the authorisation. According to the technical dossier from the applicant, the general surveillance will take into consideration, and be proportionate to, the extent of imports of T45 and use thereof in the EU Member States. In order to increase the possibility of detecting any unanticipated adverse effects, a monitoring system will be used, which involves the authorisation holder and operators handling and using viable T45. The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable seeds.

The applicant proposed to build its general surveillance on the following approaches; 1) Procedure(s) from the food/feed business operators based on the Hazard Analysis of Critical Control Point (HACCP) principles, 2) review of scientific information provided by existing monitoring network, 3) the monitoring and review of ongoing research and development, as well as scientific literature.
The scope of the monitoring plan provided by the applicant is in line with the intended uses for the event T45.

The applicant will submit an annual monitoring report covering results of the general surveillance in accordance with the conditions of the authorisation. The report will contain information of any unanticipated adverse effects that have arisen from handling and use of viable T45. According to the monitoring plan, the report will include a scientific evaluation of the confirmed adverse effect, a conclusion of the safety of T45 and, as appropriate, the measures that were taken to ensure the safety of human and animal health or the environment.

Data gaps

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

Conclusion

Molecular characterisation

The molecular characterisation data established that only one copy of the gene cassette is integrated in the oilseed rape genomic DNA. Appropriate analysis of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analyses of junction regions demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The genetic stability of transformation event T45 was demonstrated at the genomic level over multiple generations by Southern analysis. Segregation analysis shows that event T45 is inherited as dominant, single locus trait. Phenotypic stability has been confirmed by stable tolerance to the herbicide for T45 lines and varieties derived from the event grown in Canada since 1993.

Oilseed rape transformation event T45 and the physical, chemical and functional characteristics of the proteins have previously been evaluated by The VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2007a).

Comparative assessment

Based on results from comparative analyses of data from field trials located at representative sites and environments in Canada in 1995-1997, it is concluded that oilseed rape T45 is agronomically and phenotypically equivalent to the conventional counterpart and commercial available reference varieties, with the exception of the herbicide tolerance conferred by the PAT protein and maturity. The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of event T45 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glufosinate herbicide do not alter the phenotypic and agronomic characteristics of event T45 compared to conventional oilseed rape.

Environmental risk

According to the applicant, the event T45 has been phased out, and stocks of all oilseed rape T45 lines have been recalled from distribution and destroyed since 2005. However, since future cultivation in third countries and import of oilseed rape T45 into the EU/EEA area cannot be entirely ruled out, the environmental risk assessment consider exposure of viable seeds of T45 through accidental spillage into the environment during transportation, storage, handling, processing and use of derived products.

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

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

There is no evidence that the herbicide tolerant trait results in enhanced fitness, persistence or invasiveness of oilseed rape T45, or hybridizing wild relatives, compared to conventional oilseed rape varieties, unless the plants are exposed to herbicides with the active substance glufosinate ammonium. 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.

Accidental spillage and loss of viable seeds of T45 during transport, storage, handling in the environment and processing into derived products is, however, likely to take place over time, and the establishment of small populations of oilseed rape T45 cannot be excluded. Feral oilseed rape T45 arising from spilled seed could theoretically pollinate conventional crop plants if the escaped populations are immediately adjacent to field crops, and shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops.

However, both the occurrence of feral oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape populations to wild populations are likely to be low in an import scenario. Apart from the 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. 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.

Overall conclusion
Taking into account the expected limited import of oilseed rape T45 (EU COM 2009), the VKM GMO Panel considers that the routes of gene flow from T45 would not introduce significant numbers of transgenic plants into agricultural areas or result in any environmental consequences in Norway.

The VKM GMO Panel concludes that oilseed rape T45 is unlikely to have any adverse effect on the environment in Norway in the context of its intended usage.

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Appendix 1

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

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

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

Processing of rapeseed (OECD 2009)

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

1. Seed cleaning

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

2. Seed pre-conditioning and flaking

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

3. Seed cooking/conditioning

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

4. Pressing

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

5. Solvent extraction

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

6. Desolventizing of oil and meal

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

7. Degumming of oil

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

8. Alkali and physical refining of oil

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

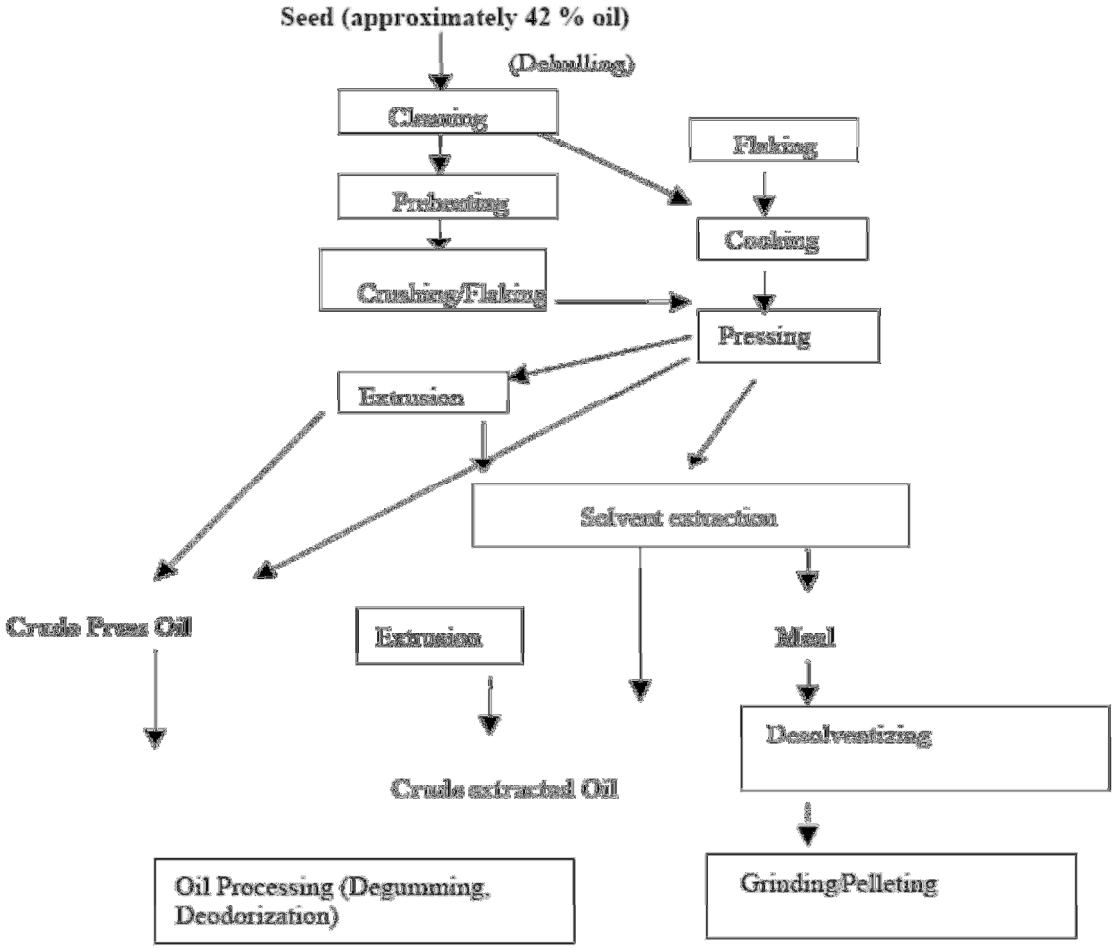

Figure 1 **Schematic illustration of the processing of low erucic acid rapeseed meal and low erucic acid rapeseed oil (OECD 2001).**

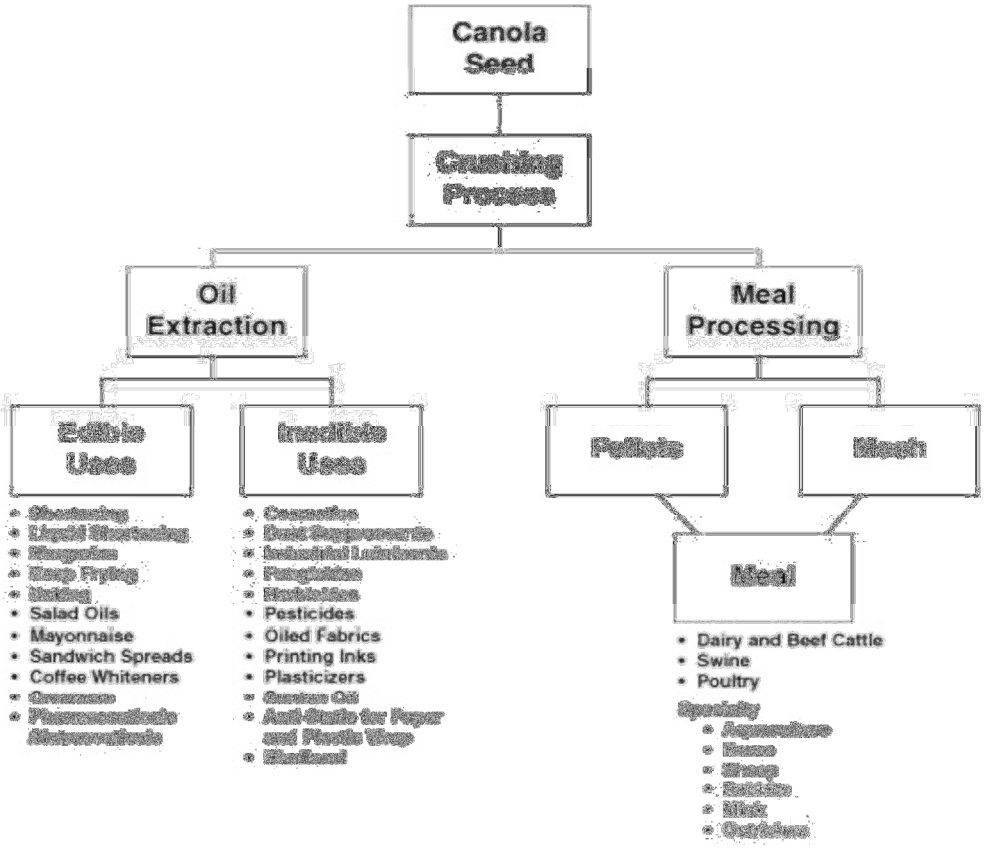

Figure 2 **Areas of application and products from processing of rapeseed (Canola Council of Canada 2005).**

Appendix 2

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

Wild turnip (*B. rapa* ssp. *campestris* (L.) A.R. Clapham)
A number of studies have shown that hybridization between *B. napus* and *B. rapa* ssp. *campestris* occurs spontaneously in the field (e.g., Jørgensen & Andersen 1994; Landbo et al. 1996; Mikkelsen et al. 1996; Jørgensen et al. 1996, 1998; Halfhill et al. 2004). Hybridization between these species can occur in both directions, but primarily arises with *B. rapa* ssp. *campestris* as the pollen donor. The hybridization frequency between these species is reported to range from 0 to 93 %, depending on experimental design, cultivar characteristics, and environmental conditions. Danish studies have shown that individual plants of *B. rapa* in crop fields with autumn oilseed rape produced an average of 265 hybrids per plant (i.e., 93 % F_1-hybrids) (Jørgensen et al. 1996). This is because *B. rapa* is an obligate out-crosser, and when isolated from other pollen sources due to experimental design there will be little competition for *B. napus* from other pollinators (Anon. 1999; Eastham & Sweet, 2002). When *B. rapa* and *B. napus* were grown at a 1:1 ratio, hybridization frequencies of 13 % and 9 % were observed, depending on whether *B. rapa* or *B. napus* was used as the parent plants. This illustrates that compatibility with pollen from *B. rapa* is higher than compatibility with *B. napus* pollen.

F_1-hybrids are triploid (2n = 29, AAC), sterile, or have reduced pollen fertility (Stace 1997; Warwick et al. 2003). The potential for dissemination to natural habitats will therefore be largely related to the introgression of transgenes into the weed population. Controlled experiments in the field or greenhouse (Jørgensen & Andersen 1994; Jørgensen et al. 1996; Mikkelsen et al. 1996) and experiments associated with commercial cultivation (Hansen et al. 2001; Warwick et al. 2003) have shown that backcrossing between F_1-hybrids and *B. rapa* ssp. *campestris* can occur spontaneously. A large number of backcrossed plants have also been shown to have high fertility. Snow et al. (1999) found that the BC_3-generation had a pollen fertility corresponding to 88-95 % and that the plants were as vigorous as pure *B. rapa* plants. Repeated backcrossing results in gradual loss of the C-chromosomes, with the exception of regions that are recombined into the A-genome (Johannessen 2004).

Extensive introgression has been reported from a mixed population of *B. napus* and *B. rapa* in organically farmed fields in Denmark, 11 years after conversion (Hansen et al. 2001). Of 102 plants analysed, only one individual was a first generation hybrid (F_1-hybrid), while almost half of the plants had specific genetic markers from both *B. napus* and *B. rapa*. Warwick et al. (2003) registered a hybridization frequency of up to 13.6 % between a weed population and cultivated oilseed rape plants in a commercial plantation in Canada. A later study by the same research group also demonstrated that transgenic hybrids have considerable potential to produce transgenic offspring through backcrossing (Halfhill et al. 2004). The frequency of backcrossing between *B. rapa* and transgenic hybrids with *Bt*-resistance was reported to be about 50 % in those cases where *B. rapa* was the pollen donor. If hybrid plants were the pollen source, backcrossing frequencies of 0.088 % and 0.060 %, respectively, were observed. After a generation of backcrossing between herbicide-resistant F_1-hybrids and *B. rapa* ssp. *campestris*, a large proportion of the offspring were found to be morphologically and cytologically identical to *B. rapa* ssp. *campestris*, and after repeated backcrossing to *B. rapa* around 10 % of BC_3-hybrids and BC_4-hybrids were reported to be resistant to herbicides (Metz et al. 1997).

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

exposed to selective pressures in the form of glyphosate treatment and reduced pollen fertility. This was demonstrated in both F_1-generations and backcrossed generations of the hybrid.
Turnip mustard is native to Norway. The species is a common weed in arable lowlands and is also widely distributed in the villages in the valleys and mountains in southern Norway and the most northerly counties (Lid & Lid 2005).

Mustard greens (leaf mustard) (*B. juncea* (L.) Czern.)
B. juncea and *B. napus* have a common set of chromosomes and are known to be sexually compatible. Hybrids have been produced by controlled crossings (Mikkelsen & Jørgensen 1997), and it is also known that the hybrids can form spontaneously under natural field conditions (Frello et al. 1995; Jørgensen et al. 1996; Liu et al. 2010. As reviewed in Devos (2009), in field plots with interplanted *B. napus* and *B. juncea* interspecific hybridization frequencies were low. In a Danish study, Jørgensen et al. (1996) reported a 3 % hybridization frequency from crossings with *B. napus* as a pollinator. Equivalent results have been reported from Canada (Bing et al. 1991; Eastham & Sweet 2002). Species hybridization can occur in both directions, but is most successful with *B. napus* as the pollen donor. The F_1-hybrid has low fertility (0 – 28 %), but expression of transgenes has been observed in the first generation after backcrossing to B. juncea (Jørgensen 1999).

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

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

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

Hoary mustard (*B. adpressa* Boiss.)
Hybridization between *B. napus* and *B. adpressa* occurs spontaneously in the field, primarily with hoary mustard as the pollen source (Lefol et al. 1996; Darmency & Fleury 2000). In one study in which *B. adpressa* and transgenic oilseed rape were planted in a ratio of 1:625, 1.5 % F_1-hybrids were registered (Lefol et al. 1996). In cases where sterile male oilseed rape was used as parent plants in a 1:1 ratio, a 70 % hybridization frequency was reported.

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

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

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

Chévre et al. 1997, 1998, 2000; Rieger et al. 2001; Warwick et al. 2003). Depending on genotype, Chévre et al. (2000) have suggested hybridization frequencies of between 10^{-7} and 10^{-5}. Corresponding estimates have been reported from field trials in Australia and Canada (Rieger et al. 2001; Warwick et al. 2003). The studies show reciprocal differences in crossings between these species. *B. napus* x *R. raphanistrum*-hybrids have chromosome numbers 2n = 37 (RrRrAC), and have a highly unstable genomic structure and low pollen vitality. In crossings where male sterile oilseed rape served as parent plants, each oilseed rape plant produced, on average, 45 hybrid seeds (Darmency et al. 1998). When these F_1-hybrids were grown in mixtures with wild radish, it was found that each hybrid produced less than one offspring. However, the fertility was improved in later backcrossings to the weed species. Stable integration of genetic material from *B. napus* into the genome of *R. raphanistrum* has not been observed (Jørgensen 1999; Eastham & Sweet 2002).

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

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

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

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

Several of the weed species in the *Brassica* complex readily form hybrids. Genetic exchange from oilseed rape to other incompatible species through a 'middle-species' (known as 'bridging'), has been the subject of several studies (OGTG 2002). In most cases, *B. juncea* is considered as a possible intermediate host. *B. napus* x *B. juncea* hybrids are, however, relatively rare, have reduced fertility, and the seed have poor germination characteristics. Crossings between *B. juncea* and *B. nigra* are not fully compatible, and any crosses between a *B. napus* hybrid and *B. nigra* will thus have less compatibility. Most studies conclude that the risk of transfer of genes between these species via mustard greens is very small (OGTG 2002). *B. rapa* is also an unlikely 'intermediate host', as the F_1-hybrids are sterile or have low fertility, and there is no form of seed dormancy.