Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Food, feed and 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 Environment Agency (former Norwegian Directorate for Nature Management) has requested the Norwegian Food Safety Authority (NFSA) to give final opinions on all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The Norwegian Food Safety Authority has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested the Norwegian Scientific Committee for Food Safety (VKM) to carry out scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorized in the European Union. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The assignment includes a scientific risk assessment of oilseed rape T45 from Bayer CropScience (Unique Identfier ACS-BNØØ8-2) for food and feed uses, import and processing.

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.

Oilseed rape T45 has previously been risk assessed by the VKM Panel on Genetically Modified Organisms (GMO), commissioned by the NFSA related to the EFSAs public hearing in 2007 (VKM 2007a).

The 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) and the selection of comparators for the risk assessment of GM plants (EFSA 2011b).

The scientific risk assessment of oilseed rape T45 include molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, comparative compositional analysis, food/feed safety assessments and environmental assessment.

It is emphasized that the VKM mandate does not include assessments of contribution to 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. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

The glufosinate ammonium-tolerant oilseed rape transformation event T45 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

For compositional analysis seeds were harvested from three field trials performed in Canada (1995, 2000 and 2004). These field trials were conducted using agronomic practices and field conditions typical of commercial oilseed rape cultivation and provided environmental situations representative of the geographical regions oilseed rape will be grown. The analytical data were statistically evaluated by analysis of difference between T45 oilseed rape and its non-transgenic parent variety AC Excel or to other comparators, derived from AC Excel.

Several of the components listed in OECDs consensus document (OECD 2011) concerning oilseed rape have not been analyzed in seed, oil or meal such as vitamin K and the antinutrient sinapine. Compositional analysis was carried out with respect to proximates, fibers, amino acids, vitamin E (alfa-, beta, gamma- and delta tocopherol, total tocopherol, minerals (phosphorus, iron, calcium, sodium, copper, magnesium, manganese, potassium and zinc), fatty acids, phytic acid and glucosinolates (alken glucosin, MSGL glucosin and indole glucosinolates). The PAT protein was detected by ELISA only in trace amounts in toasted meal from T45 oilseed rape and not detected in blended, degummed, refined, bleached and deodorized oil. The compositional analysis showed statistical differences for some of the analyzed components. However, this is not considered biological relevant because it is within the reference range from the literature.

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.

Food and feed safety assessment

The total amino acid sequence of the PAT protein was compared to that of known toxins and allergens listed in public databases. Based on these results, no evidence for any similarity to known toxic or allergenic proteins was found. An animal feeding study was performed in broiler chickens. This study showed no indications that neither the event T45 treated with glufosinate ammonium nor untreated, has adverse effects on feeding, growth or general health. To test the case of an acute exposure of the PAT protein to the circulatory system, an acute intravenous study was conducted in mice with highly purified (>95%) PAT protein, encoded by the *pat* gene (produced in *E. coli*). PAT protein, aprotinin (negative control) or melittin (positive control) were administered at dose levels of 1 and 10 mg/kg body weight. After 15 days the animals treated with the PAT protein and aprotinin at 10 mg/kg had no visible signs of systemic toxicity, in contrast to melittin which induced 100% mortality within 5 minutes at the same dose. Macroscopic examination of internal organs showed no signs of acute toxicity following treatment with PAT protein.

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 VKM GMO Panel 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

The VKM GMO Panel concludes that T45 oilseed rape, based on current knowledge, is comparable to conventional oilseed rape varieties concerning health risks with the intended usage. The GMO Panel likewise concludes that T45 is unlikely to have any adverse effect on the environment and agriculture 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, food and feed safety assessment, environmental risk assessment, import, processing, Regulation (EC) No 1829/2003, Directive 2001/18/EC

Norsk sammendrag

I forbindelse med forberedelse til implementering av EU-forordning 1829/2003 i norsk rett har Miljødirektoratet (tidligere Direktoratet for Naturforvalting) bedt Mattilsynet om vurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. På den bakgrunnen har Mattilsynet, i brev av 13. februar 2013 (ref. 2012/150202), bedt Vitenskapskomiteen for mattrygghet (VKM) om å utarbeide endelige vitenskapelige risikovurderinger av 39 GMOer og avledete produkter som inneholder eller består av genmodifiserte organismer, innen Mattilsynets sektoransvar. VKM er bedt om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelig risikovurdering. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige risikovurderingene som VKM tidligere har levert.

Oppdraget omfatter helse- og 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 transformeringsprosessen, 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 gen konstruksjon 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 antibiotika resistens.

Molekylær karakterisering
Den transgene rapslinjen T45 har fått tilført *pat* genet. Basert på søkers informasjon(integreringsplass og flankesekvenser for det integrerte transgenet, Southern blot analyser 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
Hovedkomponenter i rapsfrø, olje og mel fra tre feltforsøk i Canada (1995, 2000, 2004) er analysert for ernæringsmessige viktige komponenter. Dyrkingsområdene representerer forskjellige vekstmiljø for raps. Sammenligningene av ernæringsmessige komponenter er gjort mellom T45 og foreldrelinjen, samt kommersielt tilgjengelige rapssorter. Søker har en rimelig god beskrivelse av forsøksfeltoppsettet og hvordan prøvene er samlet inn. For feltforsøkene utført i 2000 og 2004 er det er foretatt analyser av ernæringsmessige viktige komponenter og utført statistiske undersøkelser på disse komponentene.

Flere komponenter som er listet opp i OECDs konsensusdokument for raps (OECD 2011) er ikke analysert for i frø og fôrvarer. Analyser av aske, vann, fiber, protein, karbohydrater, aminosyrer, fettsyrer, mineraler (fosfat, jern, kalium, kalsium, kobber, magnesium, mangan, natrium, sink), vitaminer (alfa-, beta-, gamma- og deltatokoferol, total tokoferol(vitamin E)), antinæringsstoffer (fytinsyre, alkenylglukosin, MSGL glukosin, indolglukosin, total glukosinolater, erukasyre). Det er også analysert for protein og aminosyreinnhold i forskjellige oljefraksjoner og spiseolje (dvs. raffinert, bleket og deodorisert rapsolje). Det ble ikke funnet proteiner eller aminosyrer i spiseoljen. En del av de analysene som er foretatt viser statistiske forskjeller. Det synes imidlertid ikke å være holdepunkter for at det er store forskjeller med hensyn på hovedkomponenter, mineraler, vitaminer, anti-næringsstoffer, aminosyrer og fettsyrer mellom modifisert og umodifisert raps.

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.

Helserisiko
Det er utført søk for aminosyresekvenshomologi for PAT-proteinet til aminosyresekvenser i databaser som inneholder sekvenser til kjente allergener og toksiner. Det er ikke funnet homologi til slike proteiner. Rapsolje er den eneste matvaren fra raps som benyttes av mennesker, og rapsolje inneholder ikke PAT protein. Allergener er analysert i henhold til EFSAs retningslinjer og FAO/WHOs anbefalte prosedyre (FAO/WHO 2001). Fôringsforsøket på broilere i 42 dager viser ingen store forskjeller mellom kontroll- og modifisert raps. Den genmodifiserte rapsen synes derfor ikke å være forskjellig fra umodifisert raps med hensyn på fôrkvalitet. Videre er det er utført en akutt toksistetsstudie på mus med PAT protein fremstilt fra genmodifisert *E. coli*. Renheten av proteinet var >95 %. Akuttoksisk forsøk ved intravenøs injeksjon med henholdsvis 1 og 10 mg aprotinin/kg (negativ kontroll), melittin (positiv kontroll) eller PAT i 15 dager viste ingen tegn på toksisk påvirkning, ved grov patologisk undersøkelse, av aprotinin og PAT. Den positive kontrollen ga 100 % mortalitet ved 10 mg/kg og null mortalitet ved 1 mg/kg. De kliniske og makroskopiske undersøkelsene viste ingen synlige tegn på akutt toksisitet hos musene som fikk PAT og aprotinin.

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
VKMs faggruppe for genmodifiserte organismer finner at oljerapsen T45, ut fra dagens kunnskap, er sammenlignbar med konvensjonell raps når det gjelder helserisiko ved den omsøkte bruken.

Faggruppen finner det lite trolig at utilsiktet frøspredning av rapslinjen T45 vil medføre effekter på miljø og landbruk i Norge.

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
MALDI-TOF	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 Food Safety Authority to conduct final food/feed and 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 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 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

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

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

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested the Norwegian Food Safety Authority to give final opinions on all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The request covers scope(s) relevant to the Gene Technology Act.

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

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

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

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

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

Assessment

1 Introduction

The 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 the 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) and the selection of comparators for the risk assessment of GM plants (EFSA 2011b).

The food/feed and 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.

It is emphasized that the VKM mandate does not include assessments of contribution to 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. These considerations are therefore not part of the risk assessment provided by the VKM 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 *E. 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, 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)
1842-2692	Derived from synthetic *E. coli* vector PiAN7 including ori ColE1
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
5275-5310	Synthetic DNA containing Left Border from *Agrobacterium tumefaciens* Ti plasmid pTiAch5 , the Left Border goes from position 5282 to 5304
5311-5840	Promoter region from the Cauliflower Mosaic Virus 35S transcript from vector pDH51
5841-5868	Synthetic polylinker sequences
5869-6420	Synthetic *pat* gene from *Streptomyces viridochromogenes*
6421-6440	Synthetic polylinker sequences
6441-6645	Terminator from the Cauliflower Mosaic Virus 35S transcript from vector pDH51
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)	Function
LB	Left border repeat	*Agrobacterium tumefaciens*	36	*Cis*-acting element for T-DNA transfer
P-35S	Promoter	Cauliflower Mosaic Virus	530	High level constitutive expression
	Polylinker sequence	Synthetic	28	Plasmid cloning site
pat	Glufosinate ammonium-tolerance *pat* gene	*Streptomyces viridochromogenes*	552	Herbicide tolerance and selectable marker
	Polylinker sequence	Synthetic	20	Plasmid cloning site
T-35S	Terminating signal	Cauliflower Mosaic Virus	205	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 only one copy 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. Only One copy 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.

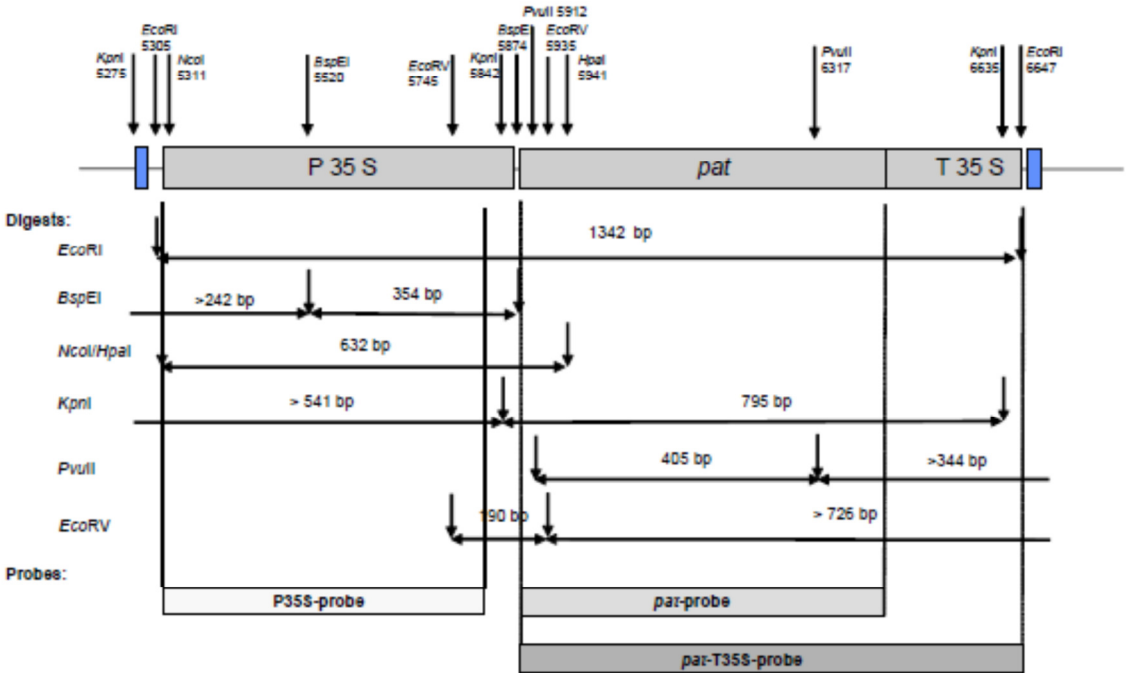

Figure 1. Schematic drawing of the T-DNA region in oilseed rape event T45

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 was 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

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.

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

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.

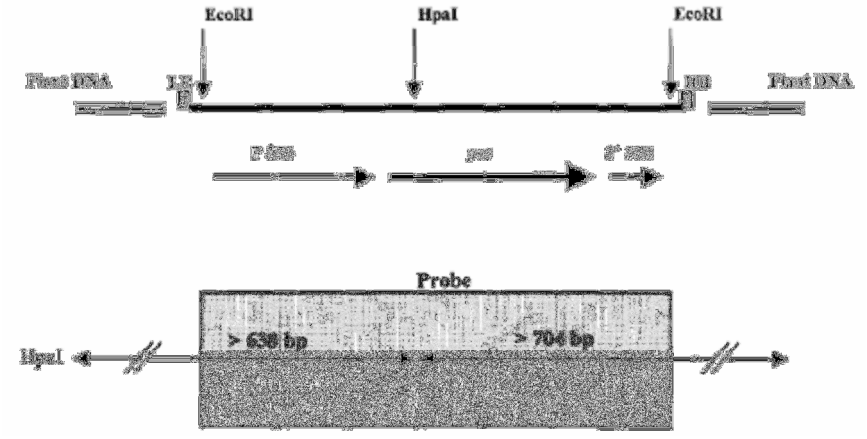

Figure 2. Schematic drawing of the hybridisation strategy

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 was integrated in the oilseed rape genomic DNA of T45. 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).

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

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

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, e.g cruciferin- and napin 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

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, 1997, 2000 and 2004.

Field trials 1995-1997
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.

Field trials 2000 and 2004
For the compositional analysis seeds were harvested from six field trials in 2000 and six field trials in 2004. Within each site, T45 sprayed with the herbicide glufosinate-ammonium and conventionally treated T45 oilseed rape was cultivated together with a non-transgenic comparator (in 2000 the oilseed rape variety AC Elect, and in 2004 Variety A sprayed with conventional herbicide). In order to cover different environmental conditions the field trials were planted on different soil types and/or 50 km

apart from each other. The plants in this study were grown under conditions typical of production practices.

In 2000, six sites were chosen and three replicates were made for each 'treatment'; T45 treated with glufosinate ammonium (495 g a.i./ha), T45 untreated and non-transgenic AC Elect untreated. Plot size was 10.5 m2. Seed was harvested at maturity.

For the 2004 season analysis material was used from replicated trials in randomized complete block design. Each site had eight plots of non-transgenic Variety A and eight of non-transgenic Variety X all conventionally treated, eight plots of T45, conventionally treated and eight plots of T45 treated with glufosinate ammonium. The plots planted with Variety X were not forwarded to compositional and nutritional analyses. The complete plots were harvested at maturity (seed moisture content below 12%) with a plot combine, except for one of the plots which were hand-harvested due to snow cover.

Results from the comparative analysis were also compared to earlier studies (Belyk 1996b; Bekyl 1996c; Belyk, 1999 [Bayer CropScience, unpublished]). In two of these studies material was collected in 1995 from three locations. All sites were seeded in 5 replicates and maintained according to agronomic practices recommended for canola in each region. Raw canola seed from transgenic and non-transgenic plots was harvested at maturity. In the third study materials was collected from three trial sites in 1994 and one in 1995 in Western Canada where canola is typically grown. The experimental design was a randomized complete block with 4 replicates. Seeding rates, fertilizer placement and weed control treatments were consistent with the local, agronomic practices.

The compositional data from 2000 and 2004 were evaluated separately. The compositional analyses were carried out by different laboratories and the analytical programs differ in some aspects and, therefore, data were not combined for statistical analysis.

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. As the data set collected in the 1996 field season represent the largest data set collected it has been pooled and subjected to ANOVA analysis, and a series of descriptive statistics are presented to further describe the mean values.

The statistical evaluation of the analytical data from the seed samples was performed based on a 95% confidence interval and a 20% bio-equivalence range. The first statistical approach taken was an extension of the t-test procedure for supporting the probability of equivalence. Instead of using the t-test table for the means comparisons to determine difference, the equivalence analysis tested whether the treatment differences exceed the range of normal variation of the non-transgenic comparator. In the second statistical approach the analysis of all composition data was repeated by means of t-tests for differences and a conclusion is drawn for the compositional and nutritional equivalence between T45 and its non-transgenic counterparts.

An analysis of variance (ANOVA) was calculated with the factors treatment, location and their interaction term. Statistical significant interaction is indicated at a 5% level ($p<0.05$), that is to say that in cases where the p-value was less than 0.05 the nutritional component measured was more influenced by the environment than the treatment (canola variety/herbicide system). Based on the ANOVA, 2-sided confidence intervals (95%) were calculated pair wise for the treatment differences (LSMEANS statement in SAS-procedure PROC MIXED). In the second statistical approach for each component the analysis was performed on a by-site basis as recommended by the authorities with analysis of variance methods (ANOVA with factor TREAT, followed by t-tests A versus B; where A, is non-transgenic control samples, B is transgenic not-liberty sprayed samples and A versus C; where C is transgenic liberty sprayed samples). In addition the components were analyzed overall sites.

The compositional analysis by Belyk (Belyk 1996b, unpublished) was performed using the one way ANOVA. Significant mean separation at a 5% level was determined by a Duncan's Multiple Range Test. Also, the amino acid data from the Belyk (Belyk, 1996c, unpublished) study were analyzed across all locations by a one way ANOVA. In the study by Belyk (Belyk, 1999, unpublished) data are compared with OECD data. Published literature was consulted for the range of values to be expected for each nutritional component (Codex Stan 210, 1999; OECD, 2001). Ranges for statistical comparison were built from the values of the non-transgenic, reference variety, AC Elect and Variety A.

4.2 Compositional analysis

For compositional analysis seeds were harvested from field trials performed at 12 different locations in Canada in 2000 and 2004. Within each site, T45 sprayed with the herbicide glufosinate-ammonium and conventionally treated T45 oilseed rape was cultivated together with a non-transgenic comparator (in 2000 the oilseed rape variety AC Elect, and in 2004 variety A) sprayed with conventional herbicide. These results were compared to earlier studies (Belyk 1996b; Belyk 1996c; Belyk 1999 [Bayer CropScience, unpublished]).

4.2.1 Proximates and fibres

For proximates and fibres the 2000 field trial data revealed lower levels of moisture, total dietary fibre, acid- and neutral detergent fibre, and crude fibre in seeds from T45 (variety Exceed) compared to non-transgenic reference AC Elect in conventional and Liberty herbicide regimes. But, in the 2004 data differences in moisture and fibre content were not observed between T45 (variety SW Flare) and non-transgenic reference Variety A in conventional and Liberty herbicide regimes. However, total- and crude fibre measurements are missing in this study. Significant differences for the majority of sites and for both treatment comparisons (A vs. B and A vs. C) can only be found for crude fibre (based on the 2000 data).

Belyk (Belyk 1996b, unpublished) reports on seed moisture, oil, protein, crude fibre, ash, phytosterol and gross energy among T45 (HCN28) and the non-transgenic varieties Excel, Legend and Cyclone, trialed at three locations in 1995. The results were not found significantly different (at the 5% level) between the varieties except for protein content of Cyclone and Legend at one location and ash content of Legend grown at another location (Appendix 2, Table 1).

In the Nutritional Impact Assessment compositional equivalence was demonstrated for the raw agricultural commodity seed generated from 12 field trial sites in 2000 and 2004. Oilseed rape was grown in the same manner as is common for commercial production, using either conventional weed control practices or treatment with glufosinate ammonium herbicide.

Statistical comparisons to test for bio-equivalence and by-site t-tests were made between glufosinate ammonium-tolerant oilseed rape and a conventional counterpart variety. The proximate and fibre compounds, amino acids, micronutrients, minerals, fatty acids, and the antinutrients phytic acid, erucic acid and glucosinolates, which are the principal components of canola oil and meal, were investigated. Further, the results were compared to earlier studies (Bekly 1996b; Bekly 1996c; Bekly 1999, unpublished) (Appendix 2, Table 1).

4.2.2 Amino acids

Only for the comparison of tyrosine data from year 2000 and between the treatments A and C the majority of the by-sites analyses shows significant differences between the non-transgenic and the transgenic plants. The over-all sites analysis for tyrosine data from year 2004 (TREAT*SITE

interactions: p>0.05) results in different findings for the two comparisons (Appendix 2, Table 2). The comparison between treatments A and B shows significant differences in tyrosine data, but this is not confirmed by the comparison between treatments A vs. C. In the year 2000 the analysis of the amino acids was performed in the oilseed rape meal after oil extraction, whereas the amino acids in the 2004 samples were measured in the seed matrix. The comparison of the determined amino acid from both studies with the literature range shows for most compounds slightly higher values. Looking at the average total protein contents of the different oilseed rape varieties tested, it becomes obvious that all mean contents are at the upper border of the literature range. Consequently, the amino acid contents after hydrolysis of the proteins are also high compared to the reference ranges for meal or seed, respectively.

Also, the amino acid composition was analyzed in the 1995 trials (Belyk 1996c, unpublished). The quantities of the amino acids in T45 (HCN28) were not significantly different from those in the control varieties except for proline and cystine to Cyclone. However, the levels fell within the range established by the commercial varieties.

Equivalence of the non-transgenics and conventionally sprayed transgenics can be assumed for all amino acids by the bioequivalence method. Comparing the non-transgenic with the Liberty sprayed and the 2 transgenic treatments amongst each other equivalence can again be stated for most of the total amino acids, only for the results for cystine, histidine, threonine and tyrosine there are treatment effects (Appendix 2, Table 4 for field studies in 2000 and 2004).

4.2.3 Tocopherols

With respect to micronutrients, beta and delta tocopherol could not be quantified in all seed samples from all sites. For alfa and total tocopherol the 2004 field trial data revealed higher levels in seeds from T45 (varity Exceed) compared to non-transgenic reference AC Elect in conventional and Liberty herbicide regimes. Significant differences with the by-site t-test analysis for the majority of sites were only found for alpha tocopherol in the comparison between treatments A versus C. This is confirmed by the over-all sites analyses performed on the alpha tocopherol results from year 2004. The determined tocopherol contents are in good compliance with literature values, so that the assessment of the year 2000 data sets is confirmed by the results from the 2004 analysis.

4.2.4 Minerals

Concerning minerals, for calcium, phosphorus, potassium, magnesium, manganese, copper and zinc, equivalence of the three treatments can be stated at almost all single sites and over all sites. Calcium, phosphorus, potassium and magnesium, for which seed reference values were found; the measured values are in good compliance with the reported ranges. For sodium and the trace elements iron, manganese, copper and zinc, the measured values fall slightly or clearly short of the range reported from literature. For sodium lower values are found for the Liberty treated samples and even lower data for conventionally treated transgenic samples compared to the non-transgenic samples. Equivalence of the three treatments could not be stated for iron and sodium; not even in the overall comparison. In some replicates of Liberty sprayed plots extraordinary high iron contents of >200 mg /kg dm were found (Appendix 2, Table 5). The reason for this might be a contamination of the samples with iron in the timeframe between harvest and analytical test in the laboratory. An analytical error can be excluded, since the high iron contents of the samples were confirmed. A second equivalence analysis was performed omitting the extreme iron values. This did not lead to a change in the by-site results, but the over-all-sites analysis between non-transgenics and conventionally sprayed transgenics resulted now in bio-equivalence. Cobalt, selenium and iodine were not analyzed.

For zinc the comparison between non-transgenic and Liberty treated transgenic samples shows for the majority of sites significant differences (Appendix 2, Table 6). This is not confirmed by the comparison between non-transgenic seeds and transgenic seed not treated with Liberty (A vs. B), but

the over-all sites analyses (TREAT*SITE interactions p>0.05) comes to the same conclusion (Appendix 2, Table 2).

4.2.5 Fatty acids

The results of the fatty acid content measured in oilseed rape seed produced in 2004 are shown in Appendix 2, Table 7. Bio-equivalence of the three treatments can be assumed for the fatty acids C16:0, C16:1, C18:1, C18:2, C20:1 and C22:0 in most of the comparisons. The results for C20:0, C20:2 and C24:1 are ambiguous, because, although the majority of the by-site comparisons shows no equivalence between the data sets, no clear tendency for a deviation from the control group can be seen (Appendix 2, Table 2). However, for all three fatty acids equivalence could be seen in the overall comparison. Equivalence could not be shown in the by-site analysis of the fatty acids C14:0, C18:0, C18:3, C22:1, and C24:0. In case of the fatty acids C14:0, C18:3 and C22:1, the transgenic samples have slightly higher amounts compared to the non-transgenic samples, whereas, for the fatty acids C18:0 and C24:0 minor findings for the transgenic samples can be observed. However, in the overall analysis equivalence could be stated for lignoceric acid (C24:0).

Values for myristic acid (C14:0) and erucic acid (C22:1) are near the limit of quantification (<0,03%) and therefore small differences in the absolute fatty acid contents lead to relatively large statistical deviations from the control mean. The absolute difference between the fatty acid mean values calculated for each site are 0.01 – 0.02 % dm. This is a difference without any nutritional relevance since the erucic acid is below 2%.

The repeated by-site t-test results for the majority of the sites resulted in significant differences between treatments for the fatty acids C16:0, C18:0, C18:2, C18:3, C20:0, and C20:2 (only between A vs. B). In these cases, the over-all sites t-tests are only valid for the fatty acid C16:0; and here the by-site findings are confirmed. These differences were small (about 10 %) except for linolenic acid levels (18:3), which were 22 % higher in T45 oilseed rape. The fatty acid values correspond very well to the data reported from literature. The non-transgenic and the transgenic oilseed rape seeds have the same fatty acid profile as commercial oilseed rape seeds currently on the market.

Fatty acid profiles in the oil and glucosinolate content in seed were also determined on material grown in 1994 and 1995 on four locations altogether (Belyk 1999, unpublished). The fatty acids analysed in the oil were: Palmitic (16:0), Palmitoleic (16:1), Stearic (18:0), Oleic (18:1), Linoleic (C18:2), Linolenic (18:3), Arachidic (20:0), Eicosenoic or Gadoleic (C20:1), Eicosadienoic (C20:2), Behenic (C22:0), Erucic (C22:1) and Lignoceric (C24:0). Fatty acids were reported as a percentage relative to the total fatty acid content in the oil. The fatty acid profile of T45 oilseed rape was compared to three commercial varieties. In this study no increase of linolenic acid was observed (Appendix 2, Table 8).

The data show substantial equivalence of the tested varieties. With the exception of a single result (the linoleic acid (18:2) content of AC Excel at Outlook in 1995), the fatty acid profiles of all varieties at all sites were within the reference range found in literature. The fatty acid profiles of all varieties at all sites were within CODEX Alimentarius standards (Codex Stan 210, 1999). In all instances erucic acid was below the 2% limit.

4.2.6 Anti-nutrients

T-tests performed on antinutrients, phytic acid, indole glucosinolates and total glucosinolates show, for the majority of sites, significant differences. But in case of phytic acid and total glucosinolates this is not stated for both treatment comparisons. Significantly increased levels of the anti-nutrients indole glucosinolates and total glucosinolates were found in both sprayed and non-sprayed T45 oilseed rape in 2004 field trial (Appendix 2, Table 5). However, the absolute difference in glucosinolate levels between the transgenic and non-transgenic oilseed rape seeds samples were small (14-17 %) in

comparison with the natural variation. Slight genetic differences between the transgenic and non-transgenic oilseed rape varieties may explain this consistent difference in glucosinolate levels. The total glucosinolate level in T45 oilseed rape was less than 16 µmol/g seed, a level which is below the threshold glucosinolate content of 25 µmol/g, set by the European Commission for certified seed of "double zero" varieties listed in the Common Catalogue of Varieties of Agricultural Plant Species (EC, 1999).

Considering glucosinolates, literature values were only found for the total glucosinolate content in oilseed rape seeds (OECD, 2001). Values measured for the non-transgenic control and the transgenic samples are all significantly below the Canadian safety threshold of 30µmol/g and the European safety threshold of 25µmol/g in air-dried seed, so that there is no concern from a safety standpoint. Additionally, all measured values are inside the range for total glucosinolate from varieties currently on the market. Even if T45 has statistically higher total glucosinolate values compared to its non-transgenic counterpart, they are comparable to the values found in other commercial oilseed rape varieties. Total glucosinolat compounds were well below the mandatory concentration for commercial canola varieties (Appendix 2, Table 8).

4.3 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.

4.3.1 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

4.3.2 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™) 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.

4.4 Conclusion

Several of the components listed in OECDs consensus document (OECD 2011) concerning oilseed rape have not been analyzed in seed, oil or meal such as vitamin K and the antinutrient sinapine. Compositional analysis was carried out with respect to proximates, fibers, amino acids, vitamin E, alfa-, beta, gamma- and delta tocopherol, total tocopherol, minerals (phosphorus, iron, calcium, sodium, copper, magnesium, manganese, potassium and zinc), fatty acids, phytic acid and glucosinolates (alken glucosin, MSGL glucosin and indole glucosinolates). The PAT protein was detected by ELISA only in trace amounts in toasted meal from T45 oilseed rape and not detected in blended, degummed, refined, bleached and deodorized oil. The compositional analysis showed statistical differences for some of the analyzed components. However, this is not considered biological relevant because it is within the reference range from the literature.

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 Food/feed safety assessment

5.1 Product description and intended uses

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. The presence of T45 oilseed rape in food and feed products is therefore expected to be restricted to adventitious levels in oilseed rape commodity, and the incidence of oilseed rape T45 in the EU/EEA expected to be limited. However, since future cultivation and import of oilseed rape T45 into the EEA area cannot be entirely ruled out, the food and feed safety assessment consider use of oilseed rape as food and feed.

The genetic modification of oilseed rape T45 is intended to improve agronomic performance only and it is not intended to influence the nutritional properties, the processing characteristics or the overall use of oilseed rape as a crop.

The scope of application EFSA/GMO/UK/2005/25 is for food and feed uses, import and processing of oilseed rape T45 and all derived products. In the human diet rapeseed is only used after processing into refined vegetable oil. The main by-product from oil processing, the mechanically and/or solvent extracted meal, is used as a protein rich feed for all classes of livestock.

5.2 Effects of processing

The effect of temperature on recombinant PAT protein encoded by the *pat* gene and produced by *E. coli* was assessed by SDS-PAGE and protein staining following incubation for up to 60 minutes at 60˚, 75˚ and 90˚C. No degradation of the PAT protein was observed under these temperature conditions. However, the PAT protein was detected by ELISA only in trace amounts in toasted meal from T45 oilseed rape and not detected in blended, degummed, refined, bleached and deodorized oil.

DNA is not present in refined oil, which is the only product intended for human consumption. The refining process for rapeseed oil also includes heating, solvent and alkali treatments that would be expected to remove and destroy DNA. The processing steps can also lead to the release of cellular enzymes (nucleases) that are responsible for degrading DNA into smaller fragments. The lack of intact DNA in the intended food products, oilseed rape oil reduces any risk of horizontal transfer of genetic material to cells in the human digestive tract as a result of the ingestion of these foods.

5.3 Toxicological assessment

The total amino acid sequence of the PAT protein (De Beuckeleer 2004b, unpublished) was compared to that of known toxins and allergens listed in 7 large public databases (SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept). The algorithm used for the homology comparison was BLASTP and the scoring matrix BLOSUM62. The criterion indicating potential toxicity or allergenicity was a 35 % identity, on a window of 80 amino acids, with a toxin or an allergen. The results of the epitope homology search showed no similarities between the PAT protein expressed by T45 and epitopes of known toxins or allergens based on a 100% identity over a linear contiguous 8 amino acid segment" matching criterion (Hérouet 2002b). Moreover, no potential glycosylation sites were identified on the PAT protein encoded by the *pat* gene. Based on these results, no evidence for any similarity to known toxic or allergenic proteins was found. As expected, the PAT protein presented only a high structural similarity with other non-toxic and non-allergenic PAT proteins (Hérouet 2002a, unpublished). The overall homology search for T45 *pat* gene product indicated significant homology only with other acetyltransferases.

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

5.3.1 Toxicological assessment of the newly expressed protein

Seed derived from T45 varieties is only different from counterpart seed by the presence of a novel protein, phosphinothricin acetyltransferase (PAT). Due to the low expression level of the PAT protein in T45 oilseed rape and the difficulties encountered in isolating a sufficient quantity of purified protein from GM oilseeds, protein safety studies were conducted with a PAT protein encoded by the *pat* gene (PAT/*pat* protein) expressed in *E. coli*. Studies were undertaken demonstrating equivalence for the

PAT protein as it is expressed in T45 and the PAT protein as it is produced by *E. coli.* Structural equivalence was demonstrated for PAT protein produced by *E. coli* and by T45 using SDS-PAGE and Western Blotting analysis. Both proteins showed indistinguishable electrophoretic mobility's and a molecular weight of approximately 22-24 kDa and can be considered equivalent. Functional equivalence between the two proteins is demonstrated by an enzymatic activity assay, showing identical substrate specificity. In addition, a glycosylation assay demonstrated that both proteins are not glycosylated, and N-terminal sequencing confirmed the identity of the proteins. Taken together, these results provide strong evidence that the protein produced in bacteria is indistinguishable from the same protein produced in plants (Currier & Hendrickx 2005, unpublished).

5.3.1.1 Acute oral toxicity testing

Acute intravenous exposure in rodents
Bayer Crop Sciences has performed an acute toxicity study of the PAT-protein in rats by a single intravenous administration. The study was performed in accordance with the principles of Good Laboratory of OECD (OECD GLP 1997)[1]. The objective of this study was to assess the acute intravenous toxicity in OF1 mice of PAT (phosphoacetyl transferase) protein (>95% purity), a protein encoded by the *pat* gene. In addition, the acute intravenous toxicity of aprotinin (negative control) and melittin (positive control) were also compared. Groups of 5 female OF1 mice were administered either with PAT protein, aprotinin or melittin in physiological saline at dose levels of 1 and 10 mg/kg body weight.

All animals were observed for clinical signs daily for fifteen days while their body weights were measured weekly. No clinical signs were noted in PAT protein-treated animals or in control groups throughout the study period. The body weight evolution was unaffected by the treatment with either PAT protein at 1 and 10 mg/kg or control substances up to Day 15. At termination of the study period, animals were subjected to a necropsy including macroscopic examination. No treatment-related macroscopic abnormalities were detected in animals treated with either PAT protein at 1 and 10 mg/kg or control substances. The positive control (melittin), at 10 mg/kg, induced 100% mortality. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 and 10 mg/kg showed no visible signs of systemic toxicity.

5.3.1.2 Sub-chronic oral toxicity testing

Repeated dose 14-day oral toxicity study in rodents.
Bayer Crop Sciences has performed a sub-chronic oral toxicity study of the PAT-protein in rats (Pfister 1996, unpublished). The study was performed in accordance with the principles of Good Laboratory of OECD (OECD GLP 1992)[2] According to the OECD guidelines the duration of exposure should normally be 28 days although a 14-day study may be appropriate in certain circumstances; justification for use of a 14-day exposure period should be provided. The duration of this repeated dose oral toxicity was 14-day exposure period. No justification for using 14-days has been found in the dossier of the applicant.

Animals of group 1 received a standard diet and rats of groups 2, 3 and 4 a low protein diet, which was adjusted to protein content similar to that of group 1 by using soya bean derived protein.

[1]Organization for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997, European Commission Directive 1999/1 I/EC, 1999, French decree n°98-1312, regarding Good Laboratory Practice, December 31, 1998, - E.P.A. (Environmental Protection Agency) • 40 CFR part 160 Federal Insecticide, Fungicide and Rodenticide Act (F1FRA): Good Laboratory Practice Standards: Final Rule, August 17, 1989, and Good Laboratory Practice Standards for Toxicology studies on Agricultural Chemicals, Ministry of Agriculture, Forestry and Fisheries (M.A.F.F.), notification 12 NohSan n°8628, (December 06 2000).

[2]Organization for Economic Cooperation and Development, Principles of Good Laboratory Practice, 1992. Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles, March 1986 and the Japanese Ministry of Agriculture, Forestry and Fisheries: On Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals, Agricultural Production Bureau, 59 NohSan Notification Number 3850, August 10, 1984. Test guidelines: The study procedures mostly conform to OECD Guidelines for Testing of Chemicals, number 407 "Repeated Dose 28-day Oral Toxicity Study in Rodents", adopted by the Council on July 27, 1995.

Protein was administered by feed admixture in powdered diet to Wistar rats of 0.5% PAT-protein + 4.5 % SOYA-protein (group 2), 5 % PAT-protein (group 3) and 5% SOYA-protein (group 4) for a period of 14 days. The study comprised four groups of each five male and five female rats. The mean intake of PAT-protein over the treatment period was: 0, 712 mg/kg body weight/day for males in group 2; 703 mg/kg body weight/day for females in group 2; 7965 mg/kg body weight/day for males in group 3 and 7619 mg/kg body weight/day for females in group 3.

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

The only changes which might be attributed to treatment were observed in clinical biochemistry parameters. They consisted of a slightly lower glucose level in males of group 4, slightly higher total cholesterol and phospholipid levels in male rats of groups 2, 3 and 4 and slightly higher triglyceride level in females of group 4 when compared with rats of group 1. Animals of group 4 received no PAT-protein but - with respect to the protein content - a diet most similar to that of groups 2 and 3. The above changes are according to the applicant considered to reflect differences in the dietary composition and to be unrelated to PAT Protein itself. Further, comparing the increased total cholesterol and phospholipid levels between group 3 (low protein diet + 5 % PAT-protein) and group 4 (low protein diet + 5 % soya protein) they are found to be in a similar range. This may suggest a similar nutritional value of both proteins.

5.3.2 Toxicological assessment of the whole GM food/feed

An animal feeding study was conducted to supplement the safety evaluation: a 42-day feeding study was performed in broiler chickens (Stafford 2005). Poultry were selected to evaluate the effects of a feed component over an entire life span and under conditions of rapid growth, thus the assay is highly sensitive for nutritional deficiencies or toxic effects. This study demonstrated equivalent performance from birds that were fed a diet containing T45 (glufosinate ammonium treated as well as untreated) as compared to a diet amended with a conventional counterpart. There were no significant mean differences in body weight gain, feed consumption, feed conversion or weights of chilled carcass, abdominal fat pad, breast, thigh, leg or wing weights among treatment groups. This study showed no indications that neither the event T45 treated with glufosinate ammonium nor untreated, has adverse effects on feeding, growth or general health.

5.4 Allergenicity assessment

5.4.1 Assessment of allergenicity of the newly expressed protein

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

An epitope sequence homology search of the PAT protein, subdivided into 8 amino acid blocks, to known epitopes belonging to known allergens has been performed. The BLASTP algorithm and the BLOSUM62 scoring matrix were also used for this search. The criterion indicating potential allergenicity was a match of at least eight contiguous identical amino acids with a known allergenic epitope. No sequence similarities with an allergenic epitope were observed (Hérouet 2002b,

unpublished). Further, an *in silico* approach enabled the search of potential N-glycosylation sites often found in allergens. The results showed that such sites of potential post-translational glycosylations were not found in the PAT protein (Hérouet 2002b, unpublished). In general, IgE binding epitopes are known to be commonly robust to treatment with heat and electroblotting on nitrocellulose. For this reason, the stability of food allergens to heat processing argues for importance of linear, continuous epitopes in assessing potential allergenicity. When treated at temperatures up to 90°C for 60 minutes, the PAT protein remains detectable by SDS-PAGE (Esdaile 2002c, unpublished). This shows the importance of the epitope homology search, which found no similarities with known allergenic epitopes. Thus, there is a reasonable certainty of no allergenicity concern associated with the presence of PAT protein.

5.4.2 Assessment of the allergenicity of the whole GM plant

Oilseed rape (*Brassica napus* L.) is not considered a common allergenic food (EC 2007). Plants are known to naturally produce toxins and allergens which often serve the plant as natural defense compounds against pests and pathogens. In the past the inclusion of oilseed rape products in human food or animal feed was limited due to the presence of some antinutrients that could act as allergenic compounds. These antinutritional and toxic factors are glucosinolates, erucic acid and phytic acid. Erucic acid is present in the oil and glucosinolates are present in the meal. Breeding efforts have reduced the levels of both erucic acid and glucosinolates resulting in "double zero" varieties (Europe) and "canola"-type varieties (Canada). In Europe, "double zero" rapeseed varieties are defined as those producing seed with a maximum glucosinolate content of 25 μmoles/g (seed weight) and with a moisture content of 9% and, having erucic acid content of not more than 2% of the total fatty acid content. Canola is defined as having less than 2% erucic acid in the oil and less than 30 μmol/g glucosinolates in the air-dried, oil-free meal. AC Excel, the recipient variety of T45, meets these criteria. The transformation process did not result in levels significantly different from the recipient variety.

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

5.4.3 Assessment of the allergenicity of proteins from the GM plant

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

Since there is considerable potential for the isolation of protein from rapeseed for use in the food industry and as an alternative to soy derivatives, milk, eggs and other plant-based and animal products, potential risk of allergic reactions to these oilseed rape proteins should have been performed by the applicant.

5.4.4 Adjuvanticity

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

5.5 Nutritional assessment of GM food/feed

5.5.1 Intake information/exposure assessment

No change in the use patterns for oilseed rape is anticipated. There is no dietary intake of PAT protein via oilseed rape products by humans, since food grade oil is the only oilseed rape product that enters the human food chain and PAT protein was not detected in any oil product derived from T45.

Nutritional assessment of rapeseed protein-A in a randomized cross-over intervention study in humans
The intervention study aimed to evaluate nutritional and physiological properties of two manufactured canola proteins with special focus on their bioavailability in humans (Fleddermann et al. 2012). The work aimed to investigate (i) the true N digestibility of the proteins in rats, (ii) the protein quality and AA distribution, and (iii) the effect of the proteins on plasma AA concentrations and N balance in humans.

Methods: 28 healthy male subjects (ø 25 years) consumed 30.0 g protein (canola protein isolate - CPI, canola protein hydrolyzate - CPH or soy protein isolate - SPI) in a randomized, double-blind, cross-over study.

On the experimental day, the volunteers had to consume a protein drink in the morning. During the rest of the 8-h investigation period, the test subjects also received two standardized low protein, carbohydrate- and fat-containing meals consisting of fruits, vegetables, gluten-free bread, butter, and jam. Subjects could selectively consume food from these meals during the 8 h. Blood samples were regularly drawn over the 8-h postprandial period and a 24-h urine sample was collected.

Results: True digestibility of the canola proteins determined in a separate rat assay showed 93.3% for CPI and 97.3% for CPH. In humans, consumption of either 30.0 g canola protein or soy protein mixed in a drink led to significant increases in plasma amino acids after 62.3 and 83.6 min, respectively.

While the CPH produced an earlier response compared to CPI and SPI, total amino acid response (AUC for 8 h) was comparable between all interventions. The nitrogen balance between the three proteins tested showed no statistical differences. Conclusions: High digestibility of rapeseed protein was found in rats. In humans, this is the first intervention study showing rapeseed protein (both isolate and hydrolyzate) as having a high nutritional quality and can be considered to be as efficient as soy protein giving an increased plasma amino acid concentration.

5.5.2 Nutritional assessment of feed derived from the GM plant

The by-products of rapeseed processing (meal and hulls) can be used in animal feed. Oilseed rape contains some toxins and anti-nutritional factors, some of which are concentrated in the meal fraction. Glucosinolates are toxins that in modern varieties are below international standards. T45 meal meets this criterion. After oil extraction with hexane the remaining meal is desolvented by heating. The

process further reduces the content of glucosinolates. The antinutritional factors include tannins, sinapine and phytic acid. Tannins and sinapine are considered to be minor antinutrients in low erucic acid, low glucosinolate oilseed rape. Although largely unavailable to the animal, phytic acid may have an impact on other mineral bioavailability (OECD 2001). With the exception of phytic acid, all of the antinutritional factors are subject to heat denaturation. Rapeseed meal is typically subjected to a moist heat treatment to facilitate oil removal. The treatment denatures proteins and detoxifies antinutrional factors.

The comparative, compositional and agronomic analyses of T45 oilseed rape and its non-GM comparators showed no biologically relevant agronomic and compositional changes in T45 oilseed rape. Therefore the GMO Panel concludes that no nutritional studies are needed (Guidance document - EFSA 2011).

5.5.3 Post-Market Monitoring of GM food/feed

The EFSA guidance document on the information needed for the risk assessment of genetically modified plants and derived food and feed has identified ten topics for consideration in a risk assessment. When Bayer CropScience evaluates food derived from T45 seed under this framework, no potential hazard was identified. Food derived from T45 does not present ethical or religious concerns and, thus, does not require special labelling beyond that required under Regulation 1830/2003. The *pat* gene is not derived from an animal, thus presenting no concerns for kosher or halal law (Regenstein, 2003). No hazard has been identified, thus there is no risk and no need for post-market monitoring.

No post-market monitoring plan is required for T45. Traditional comparators were used in the comparative analysis. The intent of the genetic modification was for agronomic benefit, no change in the nutritional composition or value was intended. No health claims are intended. Food derived from T45 will not be marketed as an alternative to or replacement for traditional rapeseed food products. T45 has no specific properties that might increase the dietary intake compared to traditional oilseed rape. There is no evidence that the long term nutritional and health status of some individuals of the European population could be impacted by the marketing of T45 derived food products.

5.6 Conclusion

No toxicity of the PAT protein was observed in a single dose acute toxicity study in mice using intravenous injection. In addition, the PAT protein is rapidly degraded under simulated gastric and intestinal conditions. The PAT protein shows no homology with known toxic proteins and/or allergens. Furthermore, the PAT protein have been extensively assessed in previous opinions of the EFSA GMO Panel, and are found to be safe (EFSA 2006c, 2007). No concerns were raised regarding the safety of the PAT protein.

The comparative analyses showed no biologically relevant agronomic or compositional changes in T45 oilseed rape, except for the introduced trait. The T45 oilseed rape is as safe as its non GM counterparts and the overall allergenicity of the whole plant is not changed through the genetic modification genetic modification. In a 42-day broiler feeding study the nutritional equivalence of T45 oilseed rape meal and conventional oilseed rape containing diets was confirmed.

Glufosinate ammonium tolerant plants containing PAT proteins already have a history of safe consumption as they have been grown widely in the USA and Canada for almost a decade without any record of adverse effects on human food or animal feed. In addition, numerous Regulatory Agencies have cleared them for human and animal consumption in many countries including Australia, Argentina, Japan, South Africa, and the European Union.

Furthermore, no toxic or allergic effect from handling T45 has been observed on workers in the field since 1993, year of its first field release.

Based on current knowledge, the VKM GMO Panel concludes that the T45 oilseed rape is as safe as its non GM counterparts for humans or animals.

6 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.

6.1 Reproduction biology of oilseed rape

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

6.2 Unintended effects on plant fitness due to the genetic modification

In natural (undisturbed) ecosystems oilseed rape is not considered to be invasive or even a significant component of any natural plant community (OECD 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.

6.3 Potential for gene transfer

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

6.3.1 Plant-to-microorganism gene transfer

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

In conclusion, the VKM GMO Panel considers it unlikely for the introduced gene in oilseed rape T45 to transfer and integrate with the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible event of transfer of the *pat* gene from T45 to soil bacteria, no novel property would be introduced into, nor expressed by the soil microbial

communities as sequence-similar genes are already present in other bacteria in soil. Therefore, no positive selective advantage that would not have been conferred by natural gene transfer between bacteria is expected.

6.3.2 Plant-to-plant gene flow

The potential for cross-pollination between oilseed rape cultivar 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.

6.3.2.1 Potential for cross-pollination with cultivated oilseed rape varieties

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

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

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

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

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

There is several plant species that are related to *B. napus* that are either cultivated, occurs as weeds of cultivated and disturbed lands, or grow in the wild outside cultivation to which gene introgression from *B. napus* could be of concern. These are found both in the *Brassica* species complex and in related genera. The following closely related species are present to varying degrees in the Norwegian flora (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 3.

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 l

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.

6.4 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.

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

The scope of this application covers import and processing, and all uses as any other oilseed rape excluding cultivation. No deliberate release of viable plant material in the EU/EEA is expected and interactions of 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.

6.6 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.

6.7 Potential interactions with the abiotic environment and biogeochemical cycles

The scope of the application covers import, processing, and food and feed use of oiseed rape 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.

6.8 Conclusion

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.

7 Data gaps

- Routes of import, transport and processing of oilseed rape seeds in Norwegian environments, and quantitative considerations of the potential of spillage.
- Established whether feral populations of oilseed rape are short-lived or have a more permanent nature. Since the places where most substantial losses occur are most likely to show the first initial populations, particularly these places should be identified and studied.
- The presence, number and viability of rape seeds in the meal and cake from the crushing process and in the waste from cleaning operations.
- No animal feeding studies have been performed on relevant production animals other than broiler chickens.

8 Conclusions

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.

Several of the components listed in OECDs consensus document (OECD 2011) concerning oilseed rape have not been analyzed in seed, oil or meal such as vitamin K and the antinutrient sinapine. Compositional analysis was carried out with respect to proximates, fibers, amino acids, vitamin E,alfa-, beta, gamma- and delta tocopherol, total tocopherol , minerals (phosphorus, iron, calcium, sodium, copper, magnesium, manganese, potassium and zinc), fatty acids, phytic acid and glucosinolates (alken glucosin, MSGL glucosin and indole glucosinolates). The PAT protein was detected by ELISA only in trace amounts in toasted meal from T45 oilseed rape and not detected in blended, degummed, refined, bleached and deodorized oil. The compositional analysis showed statistical differences for some of the analyzed components. However, these results are not considered biological relevant because they are within the reference range from the literature.

Food and feed safety assessment

The total amino acid sequence of the PAT protein was compared to that of known toxins and allergens listed in public databases. Based on these results, no evidence for any similarity to known toxic or allergenic proteins was found. An animal feeding study was performed in broiler chickens. The study showed no indications that neither the event T45 treated with glufosinate ammonium nor untreated, has adverse effects on feeding, growth or general health. To test the case of an acute exposure of the PAT protein to the circulatory system, an acute intravenous study was conducted in mice with highly purified (>95%) PAT protein, encoded by the *pat* gene (produced in *E. coli*).. PAT protein, aprotinin (negative control) or melittin (positive control) were administered at dose levels of 1 and 10 mg/kg body weight. After 15 days the animals treated with the PAT protein and aprotinin at 10 mg/kg had no visible signs of systemic toxicity, in contrast to melittin which induced 100% mortality within 5 minutes at the same dose. Macroscopic examination of internal organs showed no signs of acute toxicity following treatment with the PAT protein.

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.

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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

The VKM GMO Panel concludes that T45 oilseed rape, based on current knowledge, is comparable to conventional oilseed rape varieties concerning health risks with the intended usage. The GMO Panel likewise concludes that T45 is unlikely to have any adverse effect on the environment and agriculture 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.

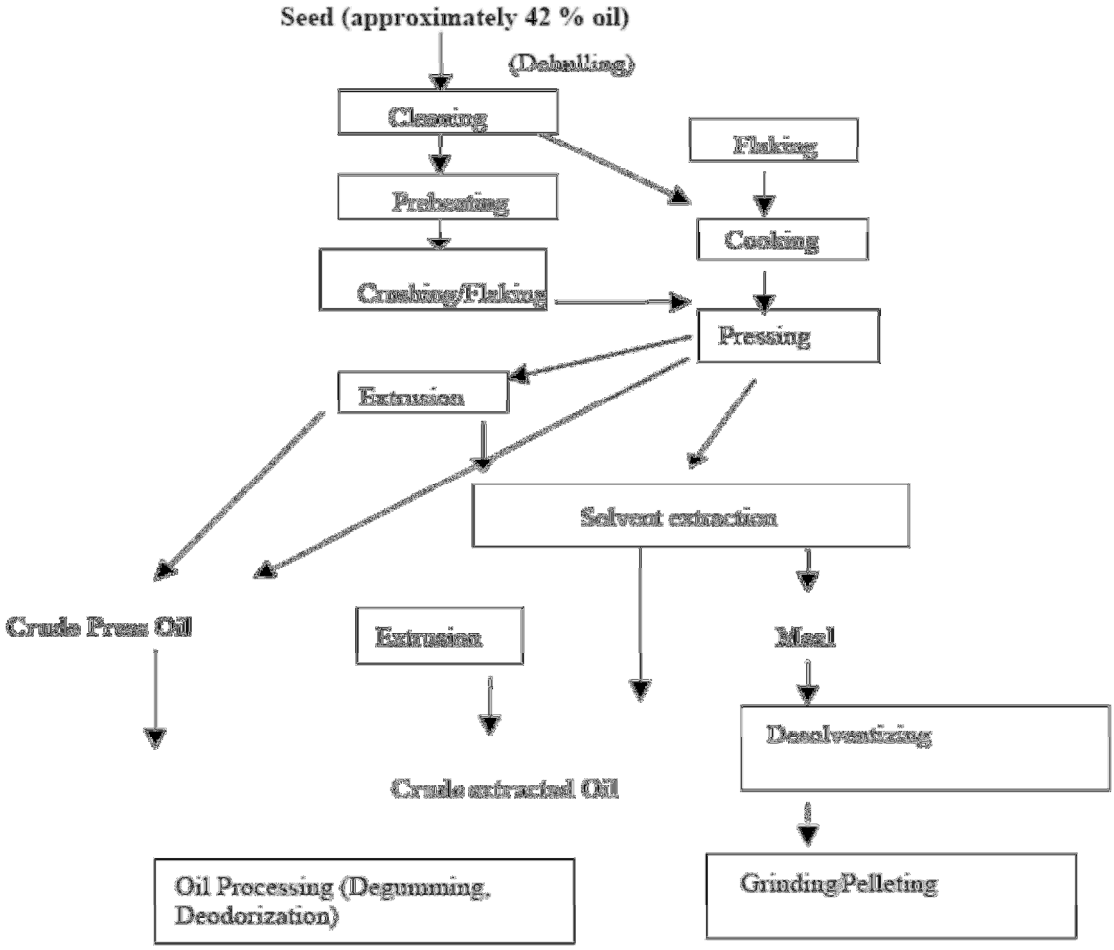

Figure 1 **Schematic illustration of the processing of low erucic acid rapeseed meal and low erucic acid rapeseed oil (OECD 2001).**

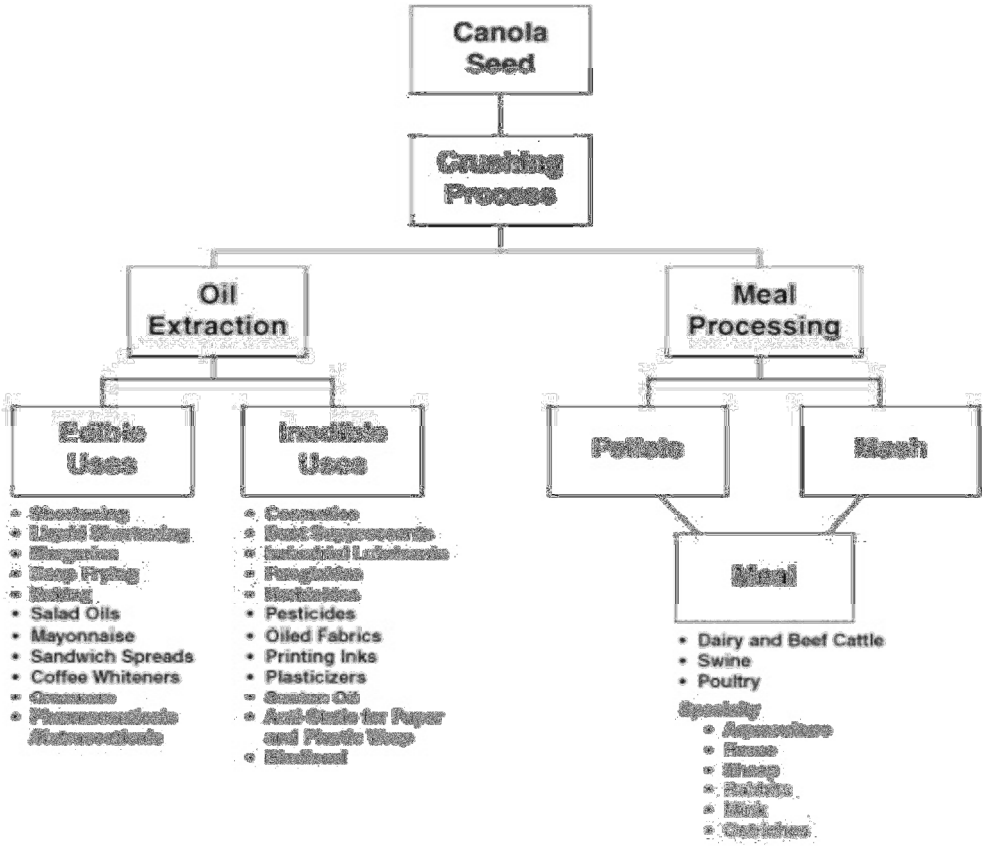

Figure 2 **Areas of application and products from processing of rapeseed (Canola Council of Canada 2005).**

Appendix 2

Table 1. Comparison of moister, oil, protein, ash, crude fibre, phytosterol contents and gross energy levels in harvested seed from transgenic *Brassica napus* lines HCN28 (T45) and innovator with non-transgenic *Brassica napus* varieties.

VIII. APPENDIX II STATISTICAL ANALYSIS RESULTS

Location	Variable	F value	P (<0.05)
High Bluff, MB	Moisture	1.69	0.1917
	Oil	0.88	0.4911
	Protein	**10.29**	**0.0001**
	Ash	0.77	0.5544
	Crude Fiber	0.54	0.7084
	Gross Energy	1.03	0.4180
Rosthern, SK	Moisture	1.31	0.3020
	Oil	0.19	0.9381
	Protein	1.03	0.4188
	Ash	**8.76**	**0.003**
	Crude Fiber	0.72	0.5875
	Gross Energy	0.23	0.9199
Indian Head, SK	Moisture	0.23	0.8711
	Oil	1.05	0.4002
	Protein	0.20	0.8978
	Ash	2.30	0.1223
	Crude Fiber	1.78	0.1968
	Gross Energy	0.91	0.4597
	Phytosterol	1.52	0.2534

Table 2. Summary of Over-all-sites ANOVA and T-test (A: non-transgenic control samples, B: transgenic not-liberty sprayed samples, C: transgenic liberty sprayed samples).

Study 00 AC 13 - Year 2000

test	mean values			p-value interaction	p-value t-test		p-value multiplicity adjusted t-test	
parameter	A	B	C	TREAT*SITE	A vs B	A vs C	A vs B	A vs C
Moisture	4.87	4.51	4.62	0.978	<.001	0.002	0.006	0.099
Fat	45.22	46.66	47.19	0.544	0.006	<.001	0.110	0.015
Protein	25.43	24.52	24.62	0.291	0.003	0.018	0.060	0.204
Ash	3.80	3.86	3.76	0.046	0.812	0.749	1.000	1.000
Carbohydrates	25.56	24.98	24.43	0.438	0.231	0.006	0.936	0.124
Total dietary fibre	16.59	14.77	15.51	0.626	<.001	<.001	<.001	0.008
Acid detergent fibre	9.59	8.85	8.47	0.214	0.029	<.001	0.271	0.018
Neutral detergent fibre	14.52	13.03	13.21	0.360	<.001	<.001	<.001	<.001
Crude fibre	10.03	9.03	8.85	0.041	<.001	<.001	<.001	<.001
alpha Tocopherol	137.9	137.0	146.1	<.001	0.701	<.001	0.973	<.001
gamma Tocopherol	306.1	287.2	287.8	0.484	<.001	<.001	0.002	0.002
Alanine	1.99	1.97	2.00	0.006	0.283	0.952	0.972	1.000
Arginine	2.87	2.82	2.99	0.002	0.240	0.005	0.923	0.025
Aspartic Acid	3.55	3.47	3.48	0.296	0.123	0.151	0.879	0.926
Cystine	0.93	0.92	1.00	0.067	0.604	<.001	1.000	0.003
Glutamic Acid	7.96	7.76	7.68	0.365	0.041	0.006	0.543	0.142
Glycine	2.30	2.26	2.18	0.073	0.034	<.001	0.375	<.001
Histidine	0.97	0.95	0.97	0.003	0.400	0.906	0.998	1.000
Isoleucine	1.81	1.77	1.81	0.042	0.169	0.901	0.900	1.000
Leucine	3.26	3.21	3.17	0.147	0.163	0.018	0.922	0.260
Lysine	2.31	2.31	2.18	0.196	0.982	<.001	1.000	<.001
Methionine	0.97	0.98	0.97	<.001	0.665	0.757	1.000	1.000
Phenylalanine	1.79	1.75	1.78	0.007	0.047	0.388	0.337	0.998
Proline	2.85	2.81	2.81	<.001	0.291	0.319	0.853	0.900
Serine	2.00	1.97	1.92	<.001	0.258	<.001	0.872	<.001
Threonine	1.84	1.84	1.79	<.001	0.957	0.281	1.000	0.437
Tryptophan	0.69	0.69	0.70	0.052	0.442	0.406	1.000	0.999
Tyrosine	1.30	1.29	1.49	0.001	0.627	<.001	1.000	<.001
Valine	2.36	2.31	2.43	0.356	0.063	0.010	0.679	0.215

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test	mean values			p-value interaction	p-value t-test		p-value multiplicity adjusted t-test	
parameter	A	B	C	TREAT*SITE	A vs B	A vs C	A vs B	A vs C
Moisture	5.88	5.73	5.79	0.295	0.002	0.163	0.027	0.904
Fat	34.77	35.05	34.20	0.260	0.806	0.026	1.000	0.231
Protein	27.29	26.73	26.72	0.837	0.095	0.090	0.692	0.705
Ash	4.62	4.67	4.84	0.102	0.343	0.014	0.983	0.163
Carbohydrates	33.32	33.55	34.16	0.143	0.272	0.010	0.983	0.085
Acid detergent fibre	9.06	9.26	9.10	0.362	0.241	0.781	0.955	1.000
Neutral detergent fibre	12.82	13.60	14.26	0.782	0.008	<.001	0.141	<.001
Calcium	0.40	0.43	0.43	0.563	<.001	<.001	<.001	0.004
Phosphorus	0.84	0.88	0.88	0.021	<.001	<.001	<.001	<.001
Potassium	0.92	0.85	0.86	0.148	<.001	<.001	<.001	0.002
Magnesium	0.36	0.38	0.38	0.148	<.001	<.001	0.008	0.001
Sodium	71.63	47.17	53.42	0.191	<.001	0.002	0.002	0.028
Iron	82.54	85.83	128.8	0.002	0.971	0.014	1.000	0.140
Manganese	32.02	33.96	35.79	0.005	0.136	<.001	0.670	0.010
Copper	3.43	3.55	3.65	0.131	0.109	0.004	0.775	0.057
Zinc	33.83	36.38	37.00	0.218	<.001	<.001	<.001	<.001
alpha Tocopherol	75.11	91.58	90.36	0.970	<.001	<.001	<.001	<.001
gamma Tocopherol	51.36	45.93	46.56	0.997	0.135	0.194	0.387	0.552
Total Tocopherols	126.4	137.5	136.9	0.994	0.021	0.028	0.109	0.124

Table 2. Summary of Over-all-sites ANOVA and T-test (continued).

test parameter	mean values A	B	C	p-value interaction TREAT*SITE	p-value t-test A vs B	A vs C	p-value multiplicity adjusted t-test A vs B	A vs C
Phytic Acid	2.21	2.45	2.38	0.010	<.001	<.001	<.001	<.001
Alkenyl Glucosinolates	8.91	9.23	9.67	0.004	0.420	0.104	0.976	0.406
MSGL Glucosinolates	0.39	0.24	0.28	0.481	<.001	<.001	<.001	<.001
Indole Glucosinolates	4.22	5.60	5.70	0.394	<.001	<.001	<.001	<.001
Total Glucosinolates	13.41	15.25	15.83	0.003	0.001	<.001	0.004	<.001
Alanine	1.24	1.19	1.21	0.462	0.005	0.175	0.089	0.957
Arginine	1.68	1.59	1.63	0.553	0.001	0.085	0.040	0.772
Aspartic Acid	2.02	1.86	1.90	0.261	<.001	<.001	<.001	0.005
Cystine	0.63	0.66	0.67	0.789	0.003	<.001	0.084	0.014
Glutamic Acid	4.73	4.64	4.72	0.085	0.170	0.842	0.846	1.000
Glycine	1.28	1.27	1.29	0.388	0.670	0.313	1.000	0.990
Histidine	0.71	0.70	0.72	0.433	0.415	0.241	0.999	0.971
Isoleucine	1.01	0.96	0.99	0.548	<.001	0.103	0.011	0.822
Leucine	1.85	1.79	1.82	0.286	0.008	0.249	0.110	0.991
Lysine	1.55	1.52	1.54	0.324	0.103	0.548	0.745	1.000
Methionine	0.52	0.51	0.51	0.138	0.250	0.416	0.964	1.000
Phenylalanine	1.09	1.03	1.06	0.068	<.001	0.007	<.001	0.135
Proline	1.72	1.68	1.72	0.854	0.247	0.891	0.984	1.000
Serine	1.12	1.09	1.10	0.051	0.155	0.393	0.801	1.000
Threonine	1.13	1.10	1.11	0.032	0.084	0.467	0.541	1.000
Tryptophan	0.37	0.36	0.36	0.764	0.179	0.149	0.941	0.919
Tyrosine	0.69	0.66	0.68	0.080	0.001	0.218	0.020	0.974
Valine	1.31	1.25	1.28	0.714	<.001	0.143	0.027	0.918
C14:0	0.04	0.04	0.04	0.457	0.049	0.076	0.561	0.801
C16:0	4.95	4.42	4.47	0.179	<.001	<.001	<.001	<.001
C16:1	0.32	0.33	0.34	0.284	0.030	0.002	0.439	0.045
C18:0	1.91	1.48	1.53	<.001	<.001	<.001	<.001	<.001
C18:1	54.21	54.86	54.69	0.007	0.059	0.211	0.497	0.978
C18:2	22.92	20.83	20.95	0.007	<.001	<.001	<.001	<.001
C18:3	11.56	14.06	13.98	0.014	<.001	<.001	<.001	<.001
C20:0	0.70	0.60	0.62	<.001	<.001	<.001	<.001	<.001
C20:1	1.56	1.62	1.61	<.001	0.007	0.016	0.069	0.140
C20:2	0.10	0.10	0.10	<.001	0.039	0.085	0.289	0.603
C22:0	0.45	0.48	0.49	0.230	0.001	<.001	0.036	0.002
C22:1	0.05	0.07	0.05	0.162	0.043	0.462	0.492	1.000
C24:0	0.25	0.21	0.23	0.517	0.002	0.063	0.051	0.745
C24:1	0.43	0.41	0.40	0.169	0.456	0.258	1.000	0.994

Table 3. Comparison of amino acids from field studies in 1995; HCN28 (T45) compared to non-transgenic Legend, Cyclone and Excel.

STAT. BASIC STATS	LSD Test; Variable: CYSTINE (4amino.sta) Marked differences are significant at p < .05000				
A	{1} M=.27575	{2} M=.26667	{3} M=.27233	{4} M=.26600	{5} M=.28050
Cyclone {1}		.214144	.003231*	.004018*	.086110
HCN92 {2}	.214144		.034577*	.042773*	.569919
HCN28 {3}	.003231*	.034577*		.919857	.108481
Excel {4}	.004018*	.042773*	.919857		.130480
Legend {5}	.086110	.569919	.108481	.130480	

STAT. BASIC STATS	LSD Test; Variable: PROLINE (4amino.sta) Marked differences are significant at p < .05000				
A	{1} M=.27575	{2} M=.26667	{3} M=.27233	{4} M=.26600	{5} M=.28050
Cyclone {1}		.498141	.039493*	.180321	.754924
HCN92 {2}	.498141		.108235	.446062	.681003
HCN28 {3}	.039493*	.108235		.379587	.048107*
Excel {4}	.180321	.446062	.379587		.245545
Legend {5}	.754924	.681003	.048107*	.245545	

Table 4. Results of By-site T-tests for Total Amino Acids

Summary t-test procedures *)		A vs. B significant	A vs. B not significant	A vs. C significant	A vs. C not significant
Alanine	2000	1	5	1	5
	2004	-	6	-	6
Arginine	2000	1	5	1	5
	2004	1	5	1	5
Aspartic Acid	2000	-	6	-	6
	2004	4	2	3	3
Cystine	2000	1	5	4	2
	2004	2	4	1	5
Glutamic Acid	2000	1	5	1	5
	2004	-	6	-	6
Glycine	2000	1	5	4	2
	2004	1	5	1	5
Histidine	2000	1	5	2	4
	2004	-	6	-	6
Isoleucine	2000	-	6	2	4
	2004	2	4	1	5
Leucine	2000	-	6	1	5
	2004	-	6	2	4
Lysine	2000	-	6	4	2
	2004	1	5	-	6
Methionine	2000	1	5	2	4
	2004	1	5	-	6
Phenylalanine	2000	1	5	2	4
	2004	5	1	3	3
Proline	2000	1	5	2	4
	2004	-	6	-	6
Serine	2000	1	5	4	2
	2004	1	5	1	5
Threonine	2000	-	6	4	2
	2004	1	5	-	6
Tryptophan	2000	-	6	1	5
	2004	-	6	-	6
Tyrosine	2000	-	6	**6**	-
	2004	1	5	1	5
Valine	2000	-	6	2	4
	2004	1	5	-	6

*) Number of sites with "S" = significant differences (p-value <0.05) or "NS" = not significant differences (p-value >0.05)
A = non-transgenic, control samples
B = transgenic, not Liberty® sprayed samples
C = transgenic, Liberty® sprayed samples

Table 5. Comparison of minerals and anti-nutrients measured in oilseed rape seed produced in 2004 from T45 (variety SW Flare) and from non-transgenic reference Variety A in conventional and in Liberty® herbicide regimes together with reference ranges for oilseed rape in commerce.

Component (on a dry matter basis)	Crop/herbicide regime			Reference Range from Literature
	Non-transgenic/ Conventional Herbicide*	Transgenic/ Conventional Herbicide*	Transgenic/ Liberty® Herbicide*	
Minerals	Mean ± SD	Mean ± SD	Mean ± SD	
Calcium %	0,40 ± 0,03	0,43 ± 0,04	0,43 ± 0,05	0,29 – 0,48
Phosphorus %	0,84 ± 0,12	0,88 ± 0,12	0,88 ± 0,15	0,48 – 0,85
Potassium %	0,92 ± 0,13	0,85 ± 0,14	0,86 ± 0,15	0,83 – 0,91
Magnesium %	0,36 ± 0,04	0,38 ± 0,05	0,38 ± 0,05	0,29 – 0,31
Sodium mg/kg [a]	71,6 ± 28,7	47,2 ± 22,3	53,4 ± 16,5	100 – 900
Iron mg/kg	82,5 ± 24,4	85,8 ± 23,8	128,8 ± 129,2 92,0 ± 25,6 [b]	160 – 640 [c]
Manganese mg/kg	32,0 ± 3,6	34,0 ± 3,0	35,8 ± 6,9	43 – 73 [c]
Copper mg/kg	3,4 ± 0,3	3,6 ± 0,3	3,6 ± 0,3	4 – 11,3 [c]
Zinc mg/kg	33,8 ± 6,3	36,4 ± 6,9	37,0 ± 7,0	43 – 77,6 [c]
Antitnutrients				
Phytic acid %	2,21 ± 0,37	2,45 ± 0,40	2,38 ± 0,44	2,0 – 5,0
Alkenyl glucosin. [d] µmol/g	8,9 ± 3,4	9,2 ± 2,9	9,7 ± 2,7	ND
MSGL glucosin. [e] µmol/g	0,4 ± 0,1	0,2 ± 0,1	0,3 ± 0,1	ND
Indole glucosin. [f] µmol/g	4,2 ± 0,7	5,6 ± 0,7	5,7 ± 0,6	ND
Total glucosinolate µmol/g	13,41 ± 3,25	15,25 ± 2,84	15,83 ± 2,96	3,8 – 33,9

[a] Conversion factor (f) from %dm to mg/kg dm f = 10 000
[b] Mean built from iron-values < 200 mg/kg dm
[c] Only values for rapeseed meal included
[d] Sum of 3-butenyl-, 4-pentenyl-, 2-hydroxy-3-butenyl- and 2-hydroxy-4-pentenyl-glucosinolate
[e] MSGL Sum of 4-methylthiobutenyl- and 5-methylthiopentenylglucosinolate
[f] Sum of indolyl-3-methyl- and 4-hydroxyindolyl-3-methylglucosinolate
ND no data

Table 6. Results of By-Site T-test for Minerales

Summary t-test procedures *)	A vs. B		A vs. C	
	Significant	not significant	Significant	not significant
Calcium	3	3	3	3
Phosphorus	3	3	3	3
Potassium	3	3	2	4
Magnesium	3	3	3	3
Sodium	2	4	2	4
Iron	-	6	1	5
Manganese	1	5	3	3
Copper	2	4	2	4
Zinc	2	4	**4**	**2**

*) Number of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences.
A = non-transgenic, control samples
B = transgenic, not Liberty® sprayed samples
C = transgenic, Liberty® sprayed samples

Table 7. Comparison of fatty acids measured in oilseed rape seed produced in 2004 from T45 (variety SW Flare) and from non-transgenic reference Variety A in conventional and in Liberty® herbicide regimes together with reference ranges for oilseed rape in commerce.

Component	Crop/herbicide regime			Reference Range from Literature
	Non-transgenic/ Conventional Herbicide*	Transgenic/ Conventional Herbicide*	Transgenic/ Liberty® Herbicide*	
Fatty acids (% relative)	Mean ± SD	Mean ± SD	Mean ± SD	
Saturated				
Myristic C14:0	0,04 ± 0,01	0,04 ± 0,01	0,04 ± 0,01	0 - 0,2
Palmitic C16:0	4,95 ± 0,42	4,42 ± 0,34	4,47 ± 0,42	2,5 - 7,0
Stearic C18:0	1,91 ± 0,34	1,48 ± 0,23	1,53 ± 0,26	0,8 - 3,0
Arachidic C20:0	0,70 ± 0,10	0,60 ± 0,08	0,62 ± 0,08	0,2 - 1,9
Behenic C22:0	0,45 ± 0,07	0,48 ± 0,08	0,49 ± 0,07	0 - 0,6
Lignoceric C24:0	0,25 ± 0,07	0,21 ± 0,06	0,23 ± 0,06	0 - 0,8
Total Saturated	8,30	7,23	7,38	3,5 - 13,5
Mono-unsaturated				
Palmitoleic C16:1	0,32 ± 0,05	0,33 ± 0,03	0,34 ± 0,04	0 - 0,6
Oleic C18:1	54,21 ± 1,98	54,86 ± 1,77	54,69 ± 1,89	50,1 - 70,0
Eicosenoic C20:1	1,56 ± 0,09	1,62 ± 0,09	1,61 ± 0,05	0,1 - 10,9
Erucic C22:1	0,05 ± 0,02	0,07 ± 0,08	0,05 ± 0,01	0 - 2,0
Nervonic C24:1	0,43 ± 0,16	0,41 ± 0,16	0,40 ± 0,08	0 - 0,4
Total Mono-unsaturated	56,14	56,88	56,69	50,2 - 83,5
Poly-unsaturated				
Linoleic C18:2	22,92 ± 1,44	20,83 ± 1,28	20,95 ± 1,26	15,0 - 30,0
Linolenic C18:3	11,56 ± 0,70	14,06 ± 0,59	13,98 ± 0,86	5,0 - 14,8
Eicosadienoic C20:2	0,10 ± 0,01	0,10 ± 0,01	0,10 ± 0,01	0 - 0,1
Total Poly-unsaturated	34,58	34,99	35,03	20 - 44,8

Table 8. Fatty acid profile and glucosinolate content of canola seed from standard commercial varieties and glufosinate tolerant T45 produced in 1994 and 1995 compared to literature data. Results (data range from 4 sites) are expressed as % of total fatty acids. Results for glucosinolates are reported as µmoles per gram meal (oil and moisture free).

Component	Non-transgenic [a]	Transgenic [b]	Reference Range from Literature [c]	Reference Range from CODEX Alimentarius [d]
Fatty acids (% relative)				
Saturated				
Palmitic C16:0	3.5 – 4.0	3.6 – 4.0	3.3 – 6.0	2.5 – 7.0
Stearic C18:0	1.6 – 2.0	1.8 – 2.0	1.1 – 2.5	0.8 – 3.0
Arachidic C20:0	0.6 – 0.7	0.6 – 0.7	0.2 – 0.8	0.2 – 1.2
Behenic C22:0	0.3 – 0.4	0.4	0.0 – 0.5	0.0 – 0.6
Lignoceric C24:0	0.2	0.2	0.0 – 0.2	0.0 – 0.3
Total Saturated	6.2 – 7.3	6.6 – 7.3	-	-
Mono-unsaturated				
Palmitoleic C16:1	0.2 – 0.3	0.2 – 0.3	0.1 – 0.6	0.0 – 0.6
Oleic C18:1	59.6 – 64.8	60.7 – 64.6	52.0 – 66.9	51.0 – 70.0
Eicosenoic C20:1	1.2 – 1.8	1.4 – 1.6	0.1 – 3.4	0.1 – 4.3
Total Mono-unsaturated	61.0 – 66.9	62.3 – 66.5	-	-
Poly-unsaturated				
Linoleic C18:2	15.8 – 20.2	16.5 – 19.4	16.1 – 24.8	15.0 – 30.0
Linolenic C18:3	8.4 – 11.0	9.2 – 10.0	6.4 – 14.1	5.0 – 14.0
Eicosadienoic C20:2	0.1	0.1	0.0 – 0.1	0.0 – 0.1
Total Poly-unsaturated	24.3 – 31.3	25.8 – 29.5	-	-
Antinutrients				
Erucic C22:1	0.0 – 0.8	0.0 – 0.1	0.0 – 2.0	0.0 – 2.0
2-hydroxy-3-butenyl glucosinolate.	5.4 – 14.0	2.3 – 8.0		
Total alkenyl-glucosinolate.	8.8 – 22.2	4.2 – 12.4	6.0 – 29.0	

[a] Range of results combined over all non-transgenic varieties at all locations
[b] Results are combined over all locations
[c] data are for low erucic acid oilseed rape as mentioned by OECD (2001)
[d] data are for low erucic acid oilseed rape, CODEX STAN 210 adopted 1999, amended 2001, 2003

Appendix 3

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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