Position papers

ACNFP advice on the safety of genetically modified 73496 oilseed rape for food and feed uses under assimilated Regulation (EC) No. 1829/2003

Reference Number RP1372

Regulated Product Dossier Assessment

Assessment finalised: 5th of April 2024

Summary

Following the submission of application RP1372 from Corteva Agrisciences LLC Represented by Corteva Agriscience UK Limited, to the Food Standards Agency (FSA) under assimilated Regulation (EC) No. 1829/2003, FSA/FSS (Food Standards Scotland) were required to undertake a safety assessment on genetically modified 73496 oilseed rape. To support the safety assessment by FSA/FSS, the Advisory Committee on Novel Foods and Processes (ACNFP) provided advice to FSA/FSS on the data submitted for the authorisation of genetically modified 73496 oilseed rape, as outlined in this document.

73496 oilseed rape is modified by the addition of the *gat*4621 (glyphosate acetyltransferase) gene cassette (a shuffled variant of three *gat* genes from *Bacillus licheniformis*). Glyphosate inhibits the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is involved in the biosynthesis of aromatic amino acids. The expression of the GAT4621 protein, encoded by the *gat*4621 gene in 73496 oilseed rape renders the crop tolerant to the herbicidal active ingredient glyphosate by acetylating its secondary amine. This generates N-acetyl glyphosate, which has no herbicidal activity.

Canada, China, the EU and India are the top oilseed rape producers with the crop also being grown in Australia, South America and the United States. The EU is the largest producer of oilseed rape. Oilseed rape seed is not commonly consumed without processing and its fractions have different uses for humans and livestock. The seed is crushed to harvest the high-quality oil, used in human diets for shallow, home or pan frying, cooking and dressing. The animal feed industry uses the by-product of the crushing process as a source of protein. The scope of the application is for the authorisation for import, processing, and food and feed use of glyphosate tolerant 73496 oilseed rape. The application does not cover cultivation and therefore no 73496 oilseed rape will be grown in the UK.

In providing its scientific advice, the ACNFP considered data provided as part of application RP1372. The molecular characterisation determined that 73496 oilseed rape contained a single, intact copy of the T-DNA at a single locus, with the presence of a disrupted triose phosphate transporter (tpt) gene near the 5' border of the insert flanking regions. From the analysis of Bioinformatic data it emerged that a fairly large rearrangement has occurred at the locus of recombination which could have caused the disruption, but from sequencing data it is not possible to confirm if this rearrangement is a consequence of the transformation event, or if it was already in the progenitor line, as the applicant does not have the parental sequence. The ACNFP concluded that it was not necessary to request parental sequencing data and that no safety concerns arose from the rearrangement. However, other tpt homologues in the oilseed rape genome are likely to compensate at least partially for the loss of the disrupted tpt gene. Moreover, as 73496 oilseed rape is commercialised as a hybrid product containing one intact and one disrupted copy of the tpt gene, the phenotypic and agronomic effect of the tpt disruption near the 73496 event is likely negligible. Bioinformatics analyses of the insert and flanking regions (including the junctions between them) found no homology with known toxic or allergenic proteins and found no sequences that could lead to horizontal gene transfer. Genetic stability of the transgenic locus, and phenotypic stability of transgenic protein expression were both confirmed. The field trials (including locations and management practices) to produce test materials for the comparative analysis were considered appropriate, and no differences between the 73496 oilseed rape and the conventional counterpart or the non-GM reference varieties that would raise safety concerns were observed. Studies on the newly expressed protein found no evidence of potential toxicology. The studies were performed using bacterially-produced protein, and the ACNFP considered that these proteins were equivalent to plant-produced proteins. Toxicological feeding studies indicate that there are no safety concerns for animal and human health. The homology searches

performed through BLASTP confirmed that GAT4621 protein shows no similarity to known allergens, toxins or antinutrients. An independent, outside contractor assessed the outcomes and methodologies of all bioinformatic analyses and was satisfied that the methods and results were satisfactory.

The ACNFP concludes that considering the nature of the introduced traits, the lack of differences in the agronomic and compositional analyses, and the proposed levels of exposure, there is no evidence that the import, processing, and food and feed use of 73496 oilseed rape would raise any safety concerns. The ACNFP concludes that 73496 oilseed rape is as safe as its conventional counterpart.

1. Introduction

1.1 Background

On September 10th 2021, the Food Standards Agency (FSA) received application RP1372 (EFSA-GMO-NL-2012-109) for the authorisation of genetically modified glyphosate tolerant 73496 oilseed rape (unique identifier: DP-Ø73496-4), submitted by Corteva Agrisciences LLC Represented by Corteva Agriscience UK Limited (European Development Centre 3B Park Square) (hereafter referred to as “the applicant”) according to Regulation (EC) No. 1829/2003, as assimilated into UK law.

The FSA and FSS checked the application for compliance with the relevant requirements of assimilated Regulation (EC) No. 1829/2003, and assimilated Regulation (EU) No. 503/2013, and on 16th December 2021, declared the application valid.

The FSA and FSS would like to thank the following members of the ACNFP who participated in the assessment: Dr Camilla Alexander-White, Dr Andy Greenfield, Dr Anton Alldrick, Alison Austin, Prof George Bassel, Dr Mark Berry, Prof Dimitris Charalampopoulos, Dr Cathrina Edwards, Prof Susan Fairweather-Tait, Prof Paul Fraser, Dr Hamid Ghouddusi, Prof Wendy Harwood, Prof Huw D. Jones, Dr Ray Kemp, Dr Elizabeth Lund, Emeritus Professor Harry J. McArdle, Rebecca McKenzie, Prof Clare Mills, Dr Lesley Stanley, Prof Hans Verhagen, Dr Maureen Wakefield, Prof Bruce Whitelaw; Dr Christine Bosch, Dr Antonio Peña-Fernández and Dr Kimon Andreas Karatzas (associate members); and Prof Pete Lund and Prof Alastair Macrae (co-opted members of ACNFP-PGT Subcommittee).

1.2 Terms of Reference

According to Articles 6 and 18 of assimilated Regulation (EC) No. 1829/2003, the FSA/\FSS were requested to carry out a scientific safety assessment of genetically modified 73496 oilseed rape for authorisation in the scope of the application, namely the import, processing, and food and feed use of 73496 oilseed rape.

FSA/FSS sought safety advice from the Advisory Committee on Novel Foods and Processes (ACNFP) on 73496 oilseed rape, which will inform the FSA/FSS safety assessment. The FSA/FSS safety assessment is to be seen as the opinion requested under Articles 6(6) and 18(6) of assimilated Regulation (EC) No. 1829/2003.

In addition to the present advice on the safety of genetically modified 73496 oilseed rape, the ACNFP was also asked to report on the particulars listed under Articles 6(5) and 18(5) of assimilated Regulation (EC) No. 1829/2003. These articles concern details that must be included in positive opinions/outcomes of assessment of GMO foods and feeds, including labelling details, any relevant conditions or restrictions, and monitoring plans.

2. Applicant details

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3. Data and methodologies

3.1 Data

The data for application RP1372 submitted according to legal requirements contained in Regulation (EC) 1829/2003 and provided by the applicant at the time of submission are specified below. To inform the FSA/FSS safety assessment of the application for renewal of the authorisation of genetically modified 73496 oilseed rape for food and feed uses in accordance with Articles 11 and 23 of Regulation (EC) No. 1829/2003, the ACNFP was asked to provide safety advice. It considered the requirements described in applicable guidance for the safety assessment of GM food and feed applications under assimilated Regulation (EC) No. 1829/2003, and based its scientific safety assessment on the data within application RP1372, additional information provided by the applicant, and any relevant peer-reviewed scientific publications.

3.2 Methodologies

The ACNFP conducted its assessment in accordance with the principles described in assimilated Regulation (EU) No. 503/2013, applicable guidance, explanatory notes, and statements (EFSA GMO Panel 2010; EFSA GMO Panel 2011; EFSA GMO Panel, 2015a; EFSA GMO Panel, 2017). Independent contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing sequencing and bioinformatics analyses.

4. Assessment

4.1 Molecular characterisation

The molecular characterisation section of the safety assessment considers the methods used to insert the transgenic material, the sequence and structure of the newly expressed protein, and the sequences at the insertion locus. Analyses performed by the applicant to determine insertion locus, copy number, and any deletions that occurred during the insertion of transgenic material are assessed. Bioinformatics analyses performed on the transgenic sequences are also assessed to ensure the newly expressed protein does not raise any safety concerns. Additionally, the expression of the new protein is assessed. Finally, bioinformatics analyses performed on the flanking regions either side of the inserted material (and the junctions between them) are assessed to ensure no sequences occur that could raise safety concerns.

4.1.1 Transformation process and vector constructs

73496 oilseed rape was produced by biolistic transformation of microspores with the *Hind III/Not I* restriction fragment, PHP28181A, from plasmid PHP28181, which contains the *Arabidopsis thaliana* polyubiquitin promoter (*UBQ10*), the *gat*4621 gene (shuffled variant of three *gat* genes from *Bacillus licheniformis*), and the terminator sequence from the proteinase inhibitor *II* gene of *Solanum tuberosum L. (pinII* terminator). To synthesize the *gat*4621 gene, three alleles of the gat gene were isolated from different strains of *Bacillus licheniformis*. *B. licheniformis* strain 401 was purchased from ATCC (catalogue number 14580); two other *B. licheniformis* strains (B6 and DS3) were isolated from soil samples and genotyped by 16S RNA sequencing. The three native *B. licheniformis* GAT enzymes were capable of acetylating glyphosate, but at a very slow rate. GAT4621 has an improved efficiency for acetylating glyphosate. A gel-purified DNA fragment isolated from plasmid PHP28181 (containing the *gat*4621 gene cassette) was used to generate 73496 oilseed rape. The expression of the GAT4621 protein, encoded by the *gat*4621 gene in 73496 oilseed rape renders the crop tolerant to the herbicidal active ingredient glyphosate by acetylating its secondary amine, generating N-acetyl glyphosate, which has no herbicidal activity.

4.1.2 Molecular studies performed on 73496 oilseed rape

Southern blot analysis confirmed that a single, intact PHP28181A DNA fragment (except for a 3 bp deletion at 5’ end) was inserted into the oilseed rape genome at a single locus in chromosome 2 and was inherited during traditional breeding processes as expected. In addition, Southern blot analysis confirmed the absence of plasmid backbone sequences in 73496 oilseed rape. The inserted PHP28181A fragment and the 5’ and 3’ flanking genomic regions were sequenced and characterised. Out of the total 6,150 bp that were sequenced, 2,003 bp were of the 5’ flanking genomic region, 2,109 bp were of the PHP28181A insertion, and 2,038 bp were of the 3’ flanking genomic region. Sequence analysis of the insertion site and flanking regions was consistent with a chromosomal rearrangement associated with the insertion site. This however does not necessarily indicate that the rearrangement is unique to oilseed rape 73496, as differences in the equivalent genomic region are also observed in at least 2 non-GMO genome assemblies. Bioinformatic analysis of the 5’ and 3’ flanking genomic regions using BLASTn/BLASTx searches against various EST (Expressed Sequence Tag) and protein datasets and the gene prediction tool FGENESH indicated the presence of a disrupted predicted triose phosphate transporter (*tpt*) gene at the 5’ flanking genomic border. Further analysis by Southern blot showed that oilseed

rape contains three or four copies of the *tpt* gene. Northern blot and qRT-PCR analysis revealed a reduced *tpt* transcription in 73496 oilseed rape plants, confirming its disruption. However, other *tpt* homologues in the oilseed rape genome appear to compensate at least partially for the loss of the disrupted *tpt* gene. Moreover, as 73496 oilseed rape is commercialised as a hybrid product containing one intact and one disrupted copy of the *tpt* gene, the phenotypic and agronomic effect of the *tpt* disruption near the 73496 event is likely negligible. Bioinformatics analyses of the insertion site found no sequences likely to contribute to horizontal gene transfer with bacterial species.

4.1.3 Transgenic protein expression

Expression levels of the GAT4621 protein were measured by a quantitative enzyme linked immunosorbent assay (ELISA) in relevant plant tissues (whole plant, root, and seed) in Conventional Herbicide Treated (CHT) and Glyphosate Treated (GT) test 73496 oilseed rape during field phase (2010) conducted at nine site locations (five sites in Canada and four sites in the United States). The inserted *gat*4621 cassette was expressed in all tissues examined. The mean GAT4621 concentration in seed was 5.6 ng/mg DW in both conventional herbicide-treated and glyphosate-treated 73496 oilseed rape; similar data are available for root and whole plant tissues in Table 1.

Table 1. Ranges and mean concentrations of GAT4621 protein in glyphosate-treated 73496 oilseed rape, conventional herbicide-treated 73496 oilseed rape, and conventional herbicide-treated control (conventional counterpart) oilseed rape, across sites, measured by a quantitative enzyme linked immunosorbent assay (ELISA).

Glyphosate-treated 73496 oilseed rape

Tissue and (Growth Stage)[a]	**ng/mg Tissue Dry Weight**		**Standard Deviation**	**Sample LLOQ[c]**	**Number of Samples**
	Mean	**Range[b]**			
Whole Plant (BBCH 15)	5.6	3.4 - 8.0	1.1	0.29	0/28

Whole Plant (BBCH 33)	4.1	2.2 - 7.2	1.1	0.29	0/32
Whole Plant (BBCH 65)	8.2	6.0 - 12	1.6	0.29	0/32
Root (BBCH 65)	4.4	1.7 - 8.7	1.8	0.22	0/32
Seed (BBCH 90)	5.6	4.2 - 8.7	1.1	0.22	0/32

Conventional[d] Herbicide-Treated 73496 Oilseed rape

Tissue and (Growth Stage)[a]	**ng/mg Tissue Dry Weight**		**Standard Deviation**	**Sample LLOQ[c]**	**Number of Samples**
	Mean	**Range[b]**			
Whole Plant (BBCH 15)	5.5	3.2 - 7.6	0.96	0.29	0/33
Whole Plant (BBCH 33)	4.4	2.6 - 6.0	0.89	0.29	0/35
Whole Plant (BBCH 65)	8.1	6.0 - 13	1.6	0.29	0/36
Root (BBCH 65)	4.2	1.6 - 7.5	1.7	0.22	0/36

Seed (BBCH 90)	5.6	3.6 - 8.1	1.1	0.22	0/36

Conventional Herbicide-Treated Control Oilseed rape

Tissue and (Growth Stage)[a]	**ng/mg Tissue Dry Weight**		**Standard Deviation**	**Sample LLOQ[c]**	**Number of Samples**
	Mean	**Range[b]**			
Whole Plant (BBCH 15)	NA[e]	0.29	NA	0.29	9/9
Whole Plant (BBCH 33)	NA	0.29	NA	0.29	9/9
Whole Plant (BBCH 65)	NA	0.29	NA	0.29	8/8
Root (BBCH 65)	NA	0.22	NA	0.22	8/8
Seed (BBCH 90)	NA	0.22	NA	0.22	9/9

[a] Adapted from Lancashire et al., (1991).

[b] Range denotes the lowest and highest individual value across sites.

[c] Lower limit of quantification (LLOQ) in ng/mg tissue dry weight.

[d] The conventional herbicide treatment consisted of sethoxydim plus clopyralid.

[e] Not applicable (NA). All samples were below the LLOQ; therefore, the mean and standard deviation were not calculated.

4.1.4 Genetic stability

Southern blot analysis confirmed the genetic stability of the insert over five generations (T2, T3, T3F2, T3F3, and F1). For each generation, the expected fragments were obtained demonstrating the structural stability of the insert. The presence of the 73496 insertion was confirmed by event-specific and *gat*4621 gene-specific endpoint PCR analyses performed on leaf punches from seedlings of each generation. The herbicide tolerance phenotype was evaluated by treating the plants with glyphosate followed by visual evaluation of herbicide injury. Chi-square analysis confirmed that the inserted DNA and the herbicide-tolerance phenotype in several self-and cross-pollination generations of 73496 oilseed rape segregate according to Mendelian principles.

4.1.5 Conclusion on the molecular characterisation

On the basis of the molecular characterisation data provided, the 73496 oilseed rape contains a single, intact PHP28181A DNA fragment (except 3 bp deletion at the 5’ end). Characterisation of the insert flanking regions revealed the presence of a disrupted *tpt* gene near the 5’ border. Bioinformatics analyses of the insert, and the flanking sequences, raised no safety concerns. The genetic stability of the insert was confirmed over five generations. The expression levels of the transgenic protein were determined using suitable methodologies, and do not cause a safety concern. Together, these analyses confirmed the intactness and stability of the inserted DNA and the expression of the GAT4621 protein and its corresponding herbicide-tolerance trait.

4.2 Comparative analysis

The purpose of the comparative analysis is to compare the GM plant with its conventional counterpart, a non-GM plant with a similar genetic background. This comparison takes two forms; firstly, a comparison of the agronomic characteristics of the plant as it grows in the field which looks at the yields derived from the plants, as well as their observable characteristics such as height and colour, and a comparison of the composition of the plant after harvest which considers the nutritional value and safety of the genetically modified plant.

4.2.1 Experimental field trail design

Test material 73496 oilseed rape, along with the non-GM conventional counterpart (near-isoline oilseed rape 5536F/5676M) and six non-GM reference Pioneer brand commercially available oilseed rape varieties (44A04, 44A89, 45H72, 45H73, 46H02, and 46A65) were grown at nine sites in 2010, (five in Canada and four in the United States). The field trial sites were selected on the

basis of their inclusion in the commercial oilseed rape-growing regions of North America.

Each site utilized a randomized complete block design and contained four blocks, each containing:

- Conventional herbicide-treated (CHT) 73496 oilseed rape
- Glyphosate-treated (GT) 73496 oilseed rape
- Near-isoline conventional herbicide-treated control oilseed rape
- Three of six conventional herbicide-treated commercial reference oilseed rape lines (44A04, 44A89, 45H72, 45H73, 46H02, and/or 46A65).

The agronomic/phenotypic data and compositional data from these field trials were analysed as specified previously in guidance provided by EFSA (EFSA GMO Panel 2010; EFSA GMO Panel 2011; EFSA GMO Panel, 2015). This includes the application of a test of difference between 73496 oilseed rape and the conventional counterpart, and a test of equivalence between 73496 oilseed rape and the non-GM reference varieties.

4.2.2 Suitability of field trials and test materials

The field trial sites were selected on the basis of their inclusion in the commercial oilseed rape growing regions of North America and represented diverse climates, soils, crop rotations, irrigation systems, and management practices. The trials were placed in prime, and marginal, oilseed rape production areas.

Meteorological data were recorded weekly, and average monthly maximum and minimum temperatures and total monthly precipitation were recorded for each site. Weather conditions were generally normal however some exceptional weather events were recorded at some sites (mostly excessive rainfall).

Test material 73496 oilseed rape and the conventional counterpart were produced following good agricultural practices and with appropriate quality assurance mechanisms. Oilseed rape seed and oilseed rape seed-derived processed fractions (un-hulled oilseed rape meal, de-hulled oilseed rape meal, and RBD oil) produced from 73496 oilseed rape, herbicide-treated 73496 oilseed rape, control oilseed rape and each of four reference oilseed rape, are fully characterised for use in regulatory studies. Each bulk seed lot, un-hulled meal lot, and de-hulled meal lot was analysed using qualitative polymerase chain reaction (PCR) assays to confirm the presence or absence of GM event 73496. Germination of 73496 oilseed rape under warm, cold, and diurnal growing conditions were comparable to that of the control oilseed rape under corresponding growing

conditions. The compositional assessment of 73496 oilseed rape was based on the analysis of seeds because the major food and feed products derive from seed.

The ACNFP is satisfied that the field trials, and the materials used in the field trials are appropriate for the comparative assessment. The geographical locations, soil conditions, meteorological conditions, and the management practices used were all considered typical of the receiving environments where 73496 oilseed rape could be grown.

4.2.3 Comparative analysis (agronomic characteristics)

Comparative analyses (i.e. difference test and equivalence test) were performed for 12 agronomic characteristics (early population, final population, seedling vigour, days to flowering, flowering duration, plant height, disease incidence, insect damage, lodging, days to maturity, shattering and yield).

Results in Table 2 show that for CHT 73496 oilseed rape, 3 agronomic characteristics were statistically significantly different from the conventional counterpart, although these were confirmed as equivalent to reference varieties.

For GT 73496 oilseed rape, 4 agronomic characteristics were statistically significantly different from the conventional counterpart, although these were confirmed as equivalent to the reference varieties.

Table 2. Comparative analysis results for 12 agronomic characteristics in 73496 oilseed rape. The table shows the number of endpoints in each category.

CHT [(c)] 73496 oilseed rape

	Test of difference[(a)]	**Test of difference[(a)]**
Test of equivalence[(b)]	Not different	Significantly different
Category I	7	3[(d)]
Category II	2	0
Category III	0	0

Category IV	0	0
Not categorised	0	0
Total endpoints	9	3

GT [(c)] 73496 oilseed rape

	Test of difference[(a)]	**Test of difference[(a)]**
Test of equivalence[(b)]	Not different	Significantly different
Category I	7	4[(e)]
Category II	1	0
Category III	0	0
Category IV	0	0
Not categorised	0	0
Total endpoints	8	4

[a] Comparison between both 73496 oilseed rape entries and the conventional counterpart

[b] The test of equivalence with the reference varieties is categorised into four different outcomes; category I (equivalence with the reference varieties is demonstrated), category II (equivalence is more likely than not), category III (equivalence is less likely than not), and category IV (non-equivalence is demonstrated). No category means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.

[c] CHT: conventional herbicide-treated 73496 oilseed rape with sethoxydim plus clopyralid; GT : glyphosate-treated 73496 oilseed rape

[d] Early population, Final population, Flowering duration

[e] Early population, Final population, Flowering duration, Plant height

4.2.4 Compositional analysis

In the comparative analysis of composition, 73496 oilseed rape seeds harvested from the field trials were analysed for nutrient composition for a total of 130 analytes (see Appendix 1 at the end of the document on page 44). Of these analytes:

- 99 constituents were selected from those recommended by the OECD (OECD, 2011)
- 5 N-acetylated amino acids (N-acetylaspartate, N-acetylglutamate, N-acetylthreonine, N-acetylglycine and N-acetylserine, hereafter referred to as NAA, NAG, NAT, NAGly and NAS) were included since the GAT4621 protein has been shown to acetylate certain amino acids
- 26 free amino acids were also analysed to evaluate a possible metabolic link to the levels of acetylated amino acids.

Due to lack of equivalence to the commercial oilseed rape lines with regards to N-acetylated amino acids, a tolerance interval was used to provide further information about the natural variability in commercial reference oilseed rape lines grown under broad environmental conditions. The statistical analysis was not applied to 28 compounds (24 analytes: 14 fatty acids, 1 vitamin, and 9 glucosinolates; 4 free amino acids: α-aminobutyric acid, cysteine, hydroxyproline, and taurine), as more than 50% of the sample values fell below the LLOQ. The statistical analysis was applied to the remaining 102 compounds, with the results summarised in Table 3.

Compositional analysis results show 44 endpoints in CHT 73496 oilseed rape and 47 endpoints in GT 73496 oilseed rape were statistically significantly different from the conventional counterpart:

- Ash and Glycine in CHT 73496 oilseed rape, and Crude Fat and Ash in GT 73496 oilseed rape fell under category III. Non-equivalence in Ash and Glycine was attributed to a large, location-dependent variation, 100-fold higher than the estimated variation in commercial genotypes although the magnitude of differences was small (within 10% difference from the control

oilseed rape and the reference). Similarly for crude fat where the location-dependent variation was more than 40-fold higher than the estimated variation in commercial genotypes. A tolerance interval was used to provide further information about the natural variability in commercial reference lines grown under broad environmental conditions. The variation among commercial reference varieties was small, estimated to be 0.000100 for ash and 0.000213 for crude fat. The applicants therefore considered that the non-equivalence outcome was a consequence of the relatively small variation in commercial genotypes, which was a major component of the equivalence interval. The estimated variation in commercial genotypes, which was a major component of the equivalence interval, was relatively small and consequently resulted in a non-equivalence outcome

- Brassicasterol in both oilseed rape entries along with ADF, and Glycine in GT 73496 oilseed rape fell under category IV. The data ranges fell within the corresponding tolerance interval confirming equivalence.
- Twelve endpoints (see Appendix 1 at the end of the document on page 44, letter [o]) in CHT 73496 oilseed rape and 11 endpoints (see Appendix 1 at the end of the document on page 44, letter [q]) in GT 73496 oilseed rape were not categorised for equivalence. For these analytes, data ranges fell within the corresponding tolerance intervals confirming equivalence in both oilseed rape entries apart from NAA, NAG and NAT, where statistically significant differences were confirmed by data exceeding the upper tolerance limit.

The remaining endpoints fell under category I and II in both oilseed rape entries.

Table 3. Comparative compositional analysis results in seeds of oilseed rape 73496. The table shows the number of endpoints in each category.

CHT [(c)] 73496 oilseed rape

	Test of difference[(a)]	**Test of difference[(a)]**
Test of equivalence[(b)]	Not different	Significantly different
Category I	41	23 [(d)]
Category II	2	6 [(f)]

Category III	0	2 (h)
Category IV	0	1 (l)
Not categorised	15 (n)	12 (o)
Total endpoints	58 Appendix 1	44 Appendix 1

GT (c) 73496 oilseed rape

	Test of difference(a)	**Test of difference(a)**
Test of equivalence(b)	Not different	Significantly different
Category I	37	23 (e)
Category II	1	8 (g)
Category III	1	2 (i)
Category IV	0	3 (m)
Not categorised	16 (p)	11 (q)
Total endpoints	55 Appendix 1	47 Appendix 1

[a] Comparison between both 73496 oilseed rape entries and the conventional counterpart

[b] The test of equivalence with the reference varieties is categorised into four different outcomes; category I (equivalence with the reference varieties is demonstrated), category II (equivalence is more likely than not), category III (equivalence is less likely than not), and category IV (non-equivalence is demonstrated). No category means that the test of equivalence was not applied

because of the lack of variation among the non-GM reference varieties.

[c] CHT: conventional herbicide-treated 73496 oilseed rape with sethoxydim plus clopyralid; GT : glyphosate-treated 73496 oilseed rape

[d,e,f,g] see Appendix 1 at the end of the document on page 44

[h] Ash and Glycine

[i] Crude Fat and Ash

[l] Brassicasterol

[m] ADF, Brassicasterol and Glycine

[n,o,p,q,] see Appendix 1 at the end of this document

Those N-acetylated amino acids showing higher concentrations were further assessed in toxicological studies, as detailed in section 4.3.4.

The comparative assessment of 22 free amino acids in both CHT and GT 73496 oilseed rape demonstrated that the concentrations of free amino acids were comparable to those in the conventional counterpart commercial reference varieties. The elevated levels of NAA, NAG and NAT did not affect the concentration of the corresponding free amino acids aspartic acid, glutamic acid and threonine.

The remaining 28 components not analysed via comparative analyses were subjected to Fisher's exact test. No statistically significant difference was observed for both oilseed rape entries.

4.2.5 Conclusion on the comparative analysis

The ACNFP assessed the field trials used to generate material for the comparative analyses and considered the locations selected were representative of commercial oilseed rape production, and that the meteorological conditions and management practices used during the field trails were appropriate.

The ACNFP also assessed the results from the comparative analysis, including all the significant differences between 73496 oilseed rape and its conventional counterpart, these data did not raise safety concerns.

4.3 Food/feed safety assessment

The food/feed safety assessment covers the likelihood that the newly expressed protein, or the whole genetically modified food or feed, will cause safety concerns when consumed by humans and/or animals. This includes looking at the concentrations of newly expressed protein in the final products that will be consumed, as well as the anticipated rates of consumption by humans and animals to understand the anticipated magnitude of exposure to any newly introduced proteins. Any toxicological or allergenic effects that can be observed and any nutritional effects that consumption of the products may cause are also assessed.

4.3.1 Effects of processing

Bulk seed samples of both CHT and GT 73596 oilseed rape were processed into typical and representative products for human and/or animal consumption. These were toasted meal from de-hulled seeds, toasted meal from un-hulled seeds, refined-bleached-deodorized (RBD) oil from de-hulled seeds and RBD oil from un-hulled seeds.

High performance liquid chromatography (HPLC) identified an increase of the total tocopherol content of the RBD oil from de-hulled seeds (518 and 563 mg/kg fresh weight in CHT and GT 73496 oilseed rape, respectively), compared to the total tocopherol content in RBD oil from the conventional counterpart. (441 mg/kg fresh weight). A similar comparison of un-hulled RBD oil revealed smaller differences in the tocopherol concentration between test and control entries (523, 555 and 494 mg/kg fresh weight in CHT 73496 oilseed rape, GT 73496 oilseed rape and the conventional counterpart, respectively). The increase occurred in the γ-tocopherol level in every treatment combination. However, the level of these tocopherols remained in the range of the Codex standard for low erucic acid rapeseed oil of 189-753 mg/kg and 430-2680 mg/kg for γ-tocopherol and total tocopherol, respectively.

High performance liquid chromatography (HPLC)/tandem mass spectrometry (LC/MS/MS) did not detect any of the analysed N-acetylated amino acids in RBD oil prepared either from de-hulled or un-hulled seeds of the test and control samples. In de-hulled and un-hulled 73496 oilseed rape toasted meal an elevated level of NAA was measured in the range of 3190 - 3690 µg/g for CHT and 3070-3480 µg/g for GT 73496 oilseed rape whilst 14.5-16.8 µg/g NAA was detected in the conventional counterpart control. This was explained by the approximately 2:1 ratio of seed to meal, meaning for each weight of seeds processed, there is a corresponding meal yield of approximately 50%. This correlates to the 1860 and 1710 µg/g NAA mean values measured in seeds of 73496 oilseed rape indicating

that processing has no effect on the stability of NAA in 73496 oilseed rape toasted meal.

The NAG concentration in toasted meal prepared from de-hulled and un-hulled seeds of CHT and GT 73496 oilseed rape showed similar correlation. The stability of N-acetylated amino acids was further addressed by the HPLC/MS/MS analyses of NAA, NAG, NAT, NAS and NAGly content of the diets prepared from 73496 oilseed rape for the 90 day rat and the 42-day broiler feeding study, showing no indications for any unexpected changes in the concentration of N-acetylated amino acids of 73496 oilseed rape seeds processed in rat or poultry diets.

The GAT4621 protein concentration was analysed by ELISA. In toasted meal prepared from de-hulled and un-hulled CHT and GT test 73496 oilseed rape and the control oilseed rape, the GAT4621 protein concentration was below the LLOQ (0.22 ng/mg Sample Dry Weight) (Table 4). RBD oil was excluded from this analysis as such oil products do not contain detectable amounts of protein.

Table 4. GAT4621 Protein Concentrations in processed oilseed rape products.

Conventional Herbicide-Treated 73496 Oilseed rape

Processed Product	**Lab Sample Number**	**GAT4621 Concentration[a] (ng/mg Sample Dry Weight)**
Toasted Meal from De-Hulled Seed	17	0.22
Toasted Meal from De-Hulled Seed	18	0.22
Toasted Meal from De-Hulled Seed	19	0.22
Processed Product	**Lab Sample Number**	**GAT4621 Concentration[a] (ng/mg Sample Dry Weight)**

Toasted Meal from De-Hulled Seed	20	0.22
Toasted Meal from De-Hulled Seed	5	0.22
Toasted Meal from De-Hulled Seed	6	0.22
Toasted Meal from De-Hulled Seed	7	0.22
Toasted Meal from De-Hulled Seed	8	0.22

Glyphosate-Treated 73496 Oilseed rape

Processed Product	**Lab Sample Number**	**GAT4621 Concentration[a] (ng/mg Sample Dry Weight)**
Toasted Meal from De-Hulled Seed	21	0.22
Toasted Meal from De-Hulled Seed	22	0.22
Toasted Meal from De-Hulled Seed	23	0.22
Toasted Meal from De-Hulled Seed	24	0.22

Toasted Meal from Un-Hulled Seed	9	0.22

Processed Product	**Lab Sample Number**	**GAT4621 Concentration[a] (ng/mg Sample Dry Weight)**
Toasted Meal from Un-Hulled Seed	10	0.22
Toasted Meal from Un-Hulled Seed	11	0.22
Toasted Meal from Un-Hulled Seed	12	0.22

Near-Isoline Control Oilseed rape

Processed Product	**Lab Sample Number**	**GAT4621 Concentration[a] (ng/mg Sample Dry Weight)**
Toasted Meal from De-Hulled Seed	13	0.22
Toasted Meal from De-Hulled Seed	14	0.22
Toasted Meal from De-Hulled Seed	15	0.22
Toasted Meal from De-Hulled Seed	16	0.22

Toasted Meal from Un-Hulled Seed	1	0.22
Toasted Meal from Un-Hulled Seed	2	0.22
Toasted Meal from Un-Hulled Seed	3	0.22
Toasted Meal from Un-Hulled Seed	4	0.22

[a] All GAT4621 concentrations were below the lower limit of quantification (LLOQ).

No errors were reported

4.3.2 Activity and stability of the newly expressed protein

The studies on the newly expressed protein were performed with GAT4621 protein produced in *E. coli,* rather than protein extracted from the plant, due to the low level of protein that can be extracted directly from the plant. Structural and functional equivalence was confirmed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine molecular weight, western blot analysis to determine identity, N-terminal amino acid sequencing and matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) to confirm the protein sequence, and N-linked glycan chain staining to demonstrate lack of glycosylation.

The GAT4621 protein expressed in 73496 oilseed rape is 147 amino acids long and has an approximate molecular weight of 18 kDa. After confirming equivalence of microbially produced GAT4621 protein to that in 73496 oilseed rape, *in vitro* analysis of the substrate specificity and kinetic activity by a continuous spectrophotometric assay showed that the microbial GAT4621 protein acetylates aspartic acid, glutamic acid, serine, threonine and glycine albeit with relatively low efficiency. Its affinity for glycine was so low that a KM value could not be estimated. The catalytic efficiency of GAT4621 on aspartate, glutamate, serine, and threonine was about 1%, 0.8%, 0.05 and 0.06%, respectively, of the activity on glyphosate. Kinetic analysis could not be completed on glyphosate

analogues as no reaction was detected with GAT4621. The GAT4621 protein did not show detectable activity with any of the other amino acids, agrochemicals or antibiotics tested.

The GAT4621 protein lost half of its acetyltransferase activity when incubated at 50°C for 15 minutes was inactivated following incubation for 15 minutes at temperatures above 53°C.

The steam-treatment and electronic cooker (both at 99–104ºC for 20 min) at the press cake extraction and toasting phases of the meal preparation resulted in no quantifiable amount of the GAT4621 protein in un-hulled and de-hulled toasted meals prepared from 73496 oilseed rape (0.22 ng/mg Sample Dry Weight). Analysis of the CHT and GT 73496 diets over the course of a sub chronic oral repeated dose 90-day feeding study demonstrated that GAT4621 protein was stable under ambient conditions of use. However, under long-term (12 weeks) refrigerated storage, its concentration in the CHT and GT 73496 oilseed rape diets decreased by 26% and 42%, respectively. The GAT4621 protein is rapidly hydrolysed (30 seconds) in SGF (Simulated gastric fluid) containing pepsin at pH 1.2, and complete digestion in SIF (Simulated intestinal fluid) (pancreatin at pH 7.5) is observed in less than five minutes.

4.3.3 Toxicological testing of the newly expresses proteins

A 28-day toxicity study was performed by dietary administration of recombinant GAT4621 in mice and no treatment-related changes were observed.

Further, to fulfil the OECD Test Guideline 407, the applicant provided an additional repeated dose 28-day oral toxicity study using rats rather than mice, justifying this choice as these can provide larger amounts of serum, given the inclusion of haematology and coagulation endpoints in the clinical pathology parameters, concluding that there were no treatment-related effects on any clinical pathology parameter evaluated in males or females at any dose level.

Bioinformatic analyses of the newly expressed GAT4621 protein (including nucleotide and amino acid sequence) revealed no similarities to known toxins and did not raise safety concerns.

4.3.4 Toxicological testing of new constituents other than the newly expressed proteins

NAA in 73496 oilseed rape revealed the largest increase of all acetylated amino acids (>500 fold), while NAG (approximately 30-fold) and NAT concentrations

(four-fold) were elevated to a smaller extent, and NAS and NAGly levels were statistically unchanged. NAA and NAG are present as a natural constituent of a broad range of foods including meat, eggs, dairy, grains, nuts, vegetables and fruits, meaning there is a history of safe exposure to low levels of NAA and NAG through the diet. The potential health implications of the elevated presence of these N-acetylated amino acids were assessed considering information on their physiological functions available from the scientific literature, their dietary exposure through a thirteen-week subchronic feeding study in rats, and in addition, toxicological studies with the use of experimental animals and *in vitro* systems.

In particular, regardless of the actual role of NAA in mediating the neuropathology of Canavan disease, toxicology studies on NAA (acute oral toxicity, 28 day repeated dose toxicity, bacterial reverse mutation, mouse bone marrow erythrocyte micronucleus, sub chronic oral repeated dose 90-day toxicity and oral two-generation reproduction studies) indicate that the presence of NAA in foodstuffs presents no hazard for neurological effects in healthy individuals or exacerbation of effects in persons with Canavan disease. Among the microscopic observations in the 90-day sub chronic oral toxicity study with NAA in rats, an increased incidence and degree of hypertrophy of acinar cells was observed in the salivary glands of both male and female rats in the 500 mg of NAA/kg body weight/day exposure group compared with the control group.

A pathology review of the histological sections of the salivary glands from male broiler chickens and untreated controls was reported, showing that although small quantitative group differences were reported by the study pathologist in the cellularity of the salivary tissue in the broiler chickens, these differences were a consequence of the normal variability of salivary gland histology and the variation of the plane of section of a dispersed gland. There were no pathological alterations to indicate any treatment related effect of N-acetyl-L-aspartic acid on chicken salivary glands.

Animal feeding studies with processed fractions prepared from seeds of 73496 oilseed rape and various toxicity studies on NAA confirm that dietary exposure to NAA does not present a risk for adverse effects.

Toxicological studies performed for NAG and NAT (acute oral toxicity, 28 day repeated dose toxicity, bacterial reverse mutation, and mouse bone marrow erythrocyte micronucleus studies) indicate that dietary exposure to NAG or NAT does not present a risk for adverse effects.

4.3.5 Toxicological testing of the whole genetically modified food or feed

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant provided a repeated dose 90-day oral toxicity study in rats, in compliance with GLP. The study did not show any toxicologically significant differences observed in rats fed a diet containing either the CHT or GT 73496 oilseed rape fractions compared with rats fed diets containing non-transgenic near-isogenic oilseed rape fractions or commercial oilseed rape fractions.

No effects on survival, clinical observation, body weights, or any of the other parameters tested were observed during the study.

4.3.6 Assessment of allergenicity

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant used a weight-of-evidence approach to assess the allergenicity potential of GAT4621 protein as no single method is sufficient to predict allergenicity (Codex Alimentarius, 2009).

Oilseed rape is not considered to be a common allergenic crop. Oilseed rape pollen may on rare occasions mono-sensitize specific individuals, particularly in those subject to extensive occupational exposure to the plant, in particular during flowering season. However, protein powder prepared from oilseed rape has a novel foods authorisation and is considered to pose a risk of causing a reaction in mustard allergic subjects due to the very close sequence similarity.

The relatively low levels of expression, lack of glycosylation and lack of thermal stability are important indicators of the lack of potential allergenicity of the newly expressed protein.

Two *in silico* searches against the FARRP12 (Food Allergy Research and Resource Program) dataset of known and putative allergens (a search for continuous, identical stretches of 8 or more amino acids in addition to an 80-mer sliding window search) found no relevant identities between the newly expressed protein and known or putative allergens. Analysis of all 174 reading frames within the 73496 oilseed rape insertion site, compared to datasets of known and putative allergens using the BLASTP algorithm returned no above threshold matches and no alignments to known or putative allergens.

In silico searches (using the criterion of 35% identity in a sliding window of 80 amino acids, in addition to the eight contiguous amino acid matches) against the COMPARE database found no identities that met the thresholds of concern

between the newly expressed protein and known allergenic proteins.

The ACNFP considered the bioinformatics analyses and found no allergenicity concerns for the newly expressed protein.

4.3.7 Anticipated intake/extent of use

In accordance with assimilated Regulation (EU) No. 503/2013, the applicant provided anticipated dietary intake of GAT4621 protein in 73496 oilseed rape by using protein expression levels in 73496 oilseed rape treated with the intended herbicide (see Table 1) and international and national food consumption data.

The conservative human exposure assessment to GAT4621 protein was performed under the assumption that 100% of all protein consumed was from 73496 oilseed rape protein isolates in the European Union. The assessment revealed 1218-fold and 566-fold margin of exposure (MOE) for adult “heavy” consumers and for children between 3 and 10 years of age respectively. The animal exposure was based on a worst case exposure scenario where dairy calves consume 100% of the protein in milk replacer from 73496 oilseed rape protein isolate. The total protein intake was estimated at 4 g/kg body weight/day containing 0.09 mg/kg body weight/day GAT4621 protein, which when compared to the NOAEL (No Observed Adverse Effect Level) revealed a 667-fold MOE.

NAA, NAG and NAT exposure through the consumption of toasted meal was assessed for multiple livestock species. Based on a hypothetical total replacement scenario, after comparing with the NOAEL from the 28-day repeated dose toxicity study for each of the target compounds, the highest calculated Daily Dietary Exposure (DDE) to NAA was identified for poultry species, ranging from 21.00 mg/kg body weight/day for layers to 43.90 mg/kg body weight/day for turkeys. A minimum MOE to NAA was approximately 19-fold for turkeys, for NAG and NAT was found to be 1187-fold and 21213-fold, respectively.

The applicant further considered protein isolates both in food and feed as another potential route of exposure. The concentrations of N-acetylated amino acids were measured in protein isolates from seeds of 73496 oilseed rape processed under potential industrial processing conditions. The human consumption was conservatively estimated based on total protein intake in the European Union assuming that 100% of the total protein comes from 73496 oilseed rape isolates. The human exposure assessment revealed a 11,290-fold MOE based on the NAA NOAEL, 5,738-fold MOE based on the NAA NOEL and 703,077-fold NAG MOE for adult “heavy” consumers and a 5,313-fold MOE based on the NAA NOAEL, 2,700-

fold MOE based on the NAA NOEL and 315,172-fold NAG MOE for children aged 3 to 10. The animal exposure was based on a worst case exposure scenario where dairy calves consume 100% of the protein in milk replacer from 73496 oilseed rape protein isolate. The assessment revealed a 6,272-fold MOE based on the NAA NOAEL, 3,188-fold MOE based on the NAA NOEL and 380,833-fold NAG MOE.

4.3.8 Nutritional assessment

In addition to the comparative compositional equivalence study of 73496 oilseed rape and the conventional counterpart with regard to the levels of nutrients and anti-nutrients, the bioavailability and biological efficacy of nutrients in the processed food and feed of 73496 oilseed rape products was assessed.

In the repeated dose 90 day rat feeding study, isonitrogenous and isocaloric diets using the de-hulled oilseed rape meals and oils were used. This study did not show any toxicologically significant differences in rats fed a diet containing either the CHT 73496 or GT 73496 oilseed rape fractions compared with rats fed diets containing non-transgenic near-isogenic oilseed rape fractions or commercial oilseed rape fractions. These results support the conclusion that oilseed rape fractions produced from oilseed rape grain harvested from plants containing event DP-Ø73496-4, unsprayed or sprayed with glyphosate, are as safe and nutritious as oilseed rape fractions produced from oilseed rape grain that does not contain event DP-Ø73496-4.

Based on the outcomes of the 42-day comparative growth broiler chicken study in which chickens were fed diets containing meal (10% in the starter and 20% in the grower diets) prepared from CHT and GT oilseed rape seed, 73496 oilseed rape was considered to be nutritionally equivalent to meal produced from non-transgenic near-isogenic control oilseed rape seed.

4.3.9 Conclusion of the food/feed safety assessment

The ACNFP assessed the food/feed safety of the genetically modified 73496 oilseed rape in terms of their toxicological potential, allergenic potential, and nutritional quality. It concluded that the newly expressed protein shared no significant identity with known toxins and allergens, and the overall allergenicity of 73496 oilseed rape was not different to conventional oilseed rape. It also assessed the food/feed safety of NAA, NAG and NAT present in 73496 oilseed rape as a result of the introduced trait. Based on the data provided, the ACNFP concluded that based on the comparative analysis and the nutritional assessment, 73496 oilseed rape is not nutritionally disadvantageous, and is as

safe as conventional oilseed rape varieties.

4.4 Environmental risk assessment and monitoring plan

4.4.1 Environmental risk assessment

The environmental risk assessment (ERA) of 73496 oilseed rape was considered by the Advisory Committee on Releases to the Environment (ACRE).

The scope of the application does not include cultivation and only covers the import, processing, and food and feed use of 73496 oilseed rape. No deliberate release of viable plant material or derived products is expected. Therefore, only accidental release of viable GM seeds or propagating material during import, transportation, storage, handling, and processing was considered.

ACRE considered the ability of 73496 oilseed rape to persist under GB environmental conditions, interactions of feral 73496 oilseed rape with the environment, and the potential for horizontal gene transfer (HGT) to the environment. ACRE concluded that 73496 oilseed rape would not raise safety concerns in the event of accidental release of viable seeds or propagating material into the environment.

ACRE’s advice is available on the GOV.UK website.

4.4.2 Post-market environmental monitoring (PMEM) Plan

The PMEM plan provided by the applicants proposes general surveillance to identify the occurrence of unanticipated adverse effects due to the unintended release of 73496 oilseed rape. Exposure (via accidental release) can be controlled by clean-up measures, and the application of current practices used for the control of any adventitious oilseed rape plants, such as manual or mechanical removal, and the application of herbicides.

General surveillance will be predominantly based on collaboration with third parties, such as operators involved in the import, handling, and processing of 73496 oilseed rape. These third parties will report any potential unanticipated adverse effects to the authorisation holder, who will investigate.

The authorisation holder will submit an annual report including results of the general surveillance and any unanticipated adverse effects. If information that confirms an adverse effect becomes available, the authorisation holder will investigate, and based on a scientific evaluation, define, and implement

management measures to protect human and animal health, or the environment, as necessary.

ACRE considered the PMEM plan provided by the applicant, in conjunction with the ERA. As the ERA did not identify potential adverse effects to the environment, it was not considered necessary for case-specific monitoring to be implemented. The proposed PMEM plan and monitoring intervals are appropriate for the intended uses of 73496 oilseed rape.

5. Overall conclusions and recommendations

To support the safety assessment by FSA/FSS, the ACNFP was asked to provide advice on the data submitted for the authorisation for import, processing, and food and feed use of genetically modified 73496 oilseed rape in accordance with assimilated Regulation (EU) No. 1829/2003. 73496 oilseed rape is modified by the addition of the *gat*4621 gene cassette. The GAT4621 protein acetylates the secondary amine of glyphosate, producing N-acetyl glyphosate which has no herbicidal activity, thereby rendering the crop tolerant to the herbicidal active ingredient.

The molecular characterisation data established that 73496 oilseed rape contains a single transgenic insert and bioinformatics analyses of this insert, and the flanking sequences, raised no safety concerns. The stability of the insert was confirmed over five generations. The expression levels of the transgenic protein in oilseed rape seeds were determined using suitable methodologies, and do not cause a safety concern.

The field trials used to generate material for the comparative analyses were deemed appropriate, and the locations selected were considered representative of commercial oilseed rape production. The meteorological conditions and management practices used during the field trails were appropriate. The ACNFP also assessed the results from the comparative analysis, including all the significant differences between 73496 oilseed rape and its conventional counterpart, and found no safety concerns when compared to reference varieties.

The food/feed safety of the newly expressed protein was assessed, and no safety concerns were raised in terms of their toxicological potential, allergenic potential, and nutritional quality. Based on the comparative analysis and the nutritional assessment, 73496 oilseed rape does not cause any nutritional concerns.

Overall, the ACNFP concludes that 73496 oilseed rape is as safe as its conventional counterpart with respect to its potential effects on human and animal health.

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7. Appendices

Appendix 1

1. List of 130 tested analytes for the comparative compositional analysis:

a) 99 “OECD” parameters analysed in grain

- 7 proximates: crude protein, crude fat, crude fiber, ADF, NDF, ash, carbohydrates
- 9 minerals: calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc,
- 18 amino acids: methionine, cystine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine and tyrosine,
- 30 fatty acids: caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), heptadecadienoic acid (C17:2), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), (9,15) isomer of linoleic acid (C18:2), linolenic acid (C18:3), γ-linolenic acid

(C18:3), nonadecanoic acid (C19:0), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), heneicosanoic acid (C21:0), behenic acid (C22:0), erucic acid (C22:1), tricosanoic acid (C23:0), lignoceric acid (C24:0), nervonic acid (C24:1).

- 11 vitamins: vitamin B1 (Thiamine), vitamin B2 (Riboflavin), vitamin B3 (Niacin), vitamin B5 (Pantothenic acid), vitamin B6 (Pyridoxine), vitamin B9 (Folic acid), α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, + total tocopherols
- 10 secondary metabolites and anti-nutrients: tannins-soluble, tannins-insoluble, phytic acid, sinapine, cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, + total sterols
- 14 glucosinolates: glucoiberin, progoitrin, epi-Progoitrin, glucoraphanin, gluconapoleiferin, gluconapin, glucoalyssin, 4- hydroxyglucobrassicin, glucobrassicinapin, glucobrassicin, gluconasturtiin, 4-methoxyglucobrassicin, neoglucobrassicin, total glucosinolates

b) 5 N-acetylated amino acids analysed in seeds.

- NAA, NAG, NAT, NAS and NAGly

c) 26 free amino acids analysed in seeds.

- alanine, α-aminobutyric acid, γ-aminobutyric acid, arginine, asparagine, aspartic acid, cystine, ethanolamine, glutamic acid, glutamine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, valine.

2. Categories reported in Table 3 for CHT [(c)] 73496 oilseed rape

Category I (equivalence demonstrated)

i. Not significantly different

Crude Fat, Carbohydrates, Myristic Acid (C14:0), Heptadecanoic Acid (C17:0), Heptadecenoic Acid (C17:1), Behenic Acid (C22:0), Lignoceric Acid (C24:0), Alanine, Arginine, Glutamic Acid, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Tyrosine, Valine, Calcium, Manganese, Copper, Potassium, Vitamin B1 (Thiamine), Vitamin B5 (Pantothenic Acid), Vitamin B9 (Folic Acid), α-Tocopherol, γ-Tocopherol, Total Tocopherols, Progoitrin, Gluconapin, Glucobrassicin, Sinapine, Stigmasterol, Total Sterols. In addition the free amino acids: Arginine, Aspartic Acid, Lysine, Methionine, Ornithine, Threonine, Tyrosine,

ii. Significantly different [d]

Crude Protein, Palmitic Acid (C16:0), Palmitoleic Acid (C16:1), Oleic Acid (C18:1), Linoleic Acid (C18:2), Linolenic Acid (C18:3), Eicosenoic Acid (C20:1), Eicosadienoic Acid (C20:2), Aspartic Acid, Tryptophan, Zinc, Vitamin B3 (Niacin), δ-Tocopherol, Total Glucosinolates, Tannins-Insoluble, Cholesterol. In addition the free amino acids: Alanine, γ-Aminobutyric Acid, Asparagine, Glutamine, Phenylalanine, Proline, Valine

Category II (equivalence more likely than not)

i. Not significantly different

Campesterol and the N-acetylated amino acid N-Acetylglycine

ii. Significantly different [f]

ADF, Stearic Acid (C18:0), Arachidic Acid (C20:0), Phosphorus, Tannins-Soluble. In addition the free amino acid Serine

Category III (equivalence less likely than not)

i. Not significantly different

None

ii. Significantly different [h]

Ash. In addition the free amino acid Glycine

Category IV (non-equivalence demonstrated)

i. Not significantly different

None

ii. Significantly different [l]

Brassicasterol

Not categorised

i. Not significantly different

Tricosanoic Acid (C23:0), Nervonic Acid (C24:1), Cystine, Glycine, Phenylalanine, Serine, Threonine, Iron, Sodium, Vitamin B2 (Riboflavin), β-Sitosterol, the N-acetylated amino N-Acetylserine. In addition the free amino acids: Ethanolamine,

Glutamic Acid, Tryptophan

ii. Significantly different[o]

Crude Fiber, NDF, Magnesium, Vitamin B6 (Pyridoxine), 4-Hydroxyglucobrassicin, Phytic Acid, the N-acetylated amino acids N-Acetylaspartate, N-Acetylglutamate, N-Acetylthreonine. In addition the free amino acids Histidine, Isoleucine, Leucine

3. Categories reported in Table 3 for **GT [(c)] 73496 oilseed rape**

Category I (equivalence demonstrated)

i. Not significantly different

Carbohydrates, Myristic Acid (C14:0), Heptadecanoic Acid (C17:0), Heptadecenoic Acid (C17:1), Eicosenoic Acid (C20:1), Behenic Acid (C22:0), Lignoceric Acid (C24:0), Arginine, Histidine, Isoleucine, Lysine, Methionine, Proline, Tyrosine, Calcium, Copper, Potassium, Vitamin B1 (Thiamine), Vitamin B9 (Folic Acid), α-Tocopherol, γ-Tocopherol, Total Tocopherols, Progoitrin, Gluconapin, Glucobrassicin, Sinapine, Stigmasterol, Total Sterols. In addition the free amino acids: Arginine, Asparagine, Aspartic Acid, Glutamine, Lysine, Methionine, Ornithine, Threonine, Tyrosine.

ii. Significantly different [e]

Palmitic Acid (C16:0), Palmitoleic Acid (C16:1), Oleic Acid (C18:1), Linoleic Acid (C18:2), Linolenic Acid (C18:3), Eicosadienoic Acid (C20:2), Alanine, Aspartic Acid, Glutamic Acid, Leucine, Valine, Phosphorus, Zinc, Vitamin B3 (Niacin), Vitamin B5 (Pantothenic Acid), δ-Tocopherol, Total Glucosinolates, Tannins-Soluble, Tannins-Insoluble, Cholesterol. In addition the free amino acids: Alanine, Proline, Valine.

Category II (equivalence more likely than not)

i. Not significantly different

Campesterol

ii. Significantly different [g]

Crude Protein, Stearic Acid (C18:0), Arachidic Acid (C20:0), Tryptophan, Manganese. In addition the free amino acids: γ-Aminobutyric Acid, Phenylalanine, Serine

Category III (equivalence less likely than not)

i. Not significantly different

The N-acetylated amino acid N-Acetylglycine

ii. Significantly different [i]

Crude Fat, Ash

Category IV (non-equivalence demonstrated)

i. Not significantly different

None

ii. Significantly different [m]

ADF, Brassicasterol. In addition the free amino acid Glycine

Not categorised

i. Not significantly different [p]

Tricosanoic Acid (C23:0), Nervonic Acid (C24:1), Cystine, Glycine, Phenylalanine, Serine, Threonine, Iron, Sodium, Vitamin B2 (Riboflavin), Vitamin B6 (Pyridoxine), β-Sitosterol, the N-acetylated amino acid N-Acetylserine. In addition the free amino acids: Ethanolamine, Glutamic Acid, Tryptophan

ii. Significantly different [q]

Crude Fiber, NDF, Magnesium, 4-Hydroxyglucobrassicin, Phytic Acid, the N-acetylated amino acids N-Acetylaspartate, N-Acetylglutamate, N-Acetylthreonine. In addition the free amino acids: Histidine, Isoleucine, Leucine

Abbreviations

Acronym	Definition
ACNFP	Advisory Committee on Novel Foods and Processes
ACRE	Advisory Committee on Releases to the Environment
ADF	Acid Detergent Fiber

BLAST	Basic Local Alignment Search Tool
bp	Base pair
bw	Body weight
CD	Coeliac disease
CHT	Conventional Herbicide Treated
COMPARE	COMprehensive Protein Allergen REsource
DDE	Daily Dietary Exposure
DNA	Deoxyribonucleic acid
DW	Dry weight
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EST	Expressed Sequence Tag
EU	European Union
FARRP	Food Allergy Research and Resource Program
FSA	Food Standards Agency

FSS	Food Standard Scotland
gDNA	Genomic DNA
GM	Genetically modified
GMO	Genetically modified organism
GAT4621	Glyphosate AcetylTransferase protein
gat4621	Glyphosate AcetylTransferase gene
GCN5	General control non-depressible 5 (GCN5)
GT	Glyphosate Treated
HPLC	High performance liquid chromatography
kDa	Kilodalton
KM	Michaelis-Menten constant
LLOQ	Lower limit of quantification
MALDI-MS	Matrix Assisted Laser Desorption Ionization Mass Spectrometry
LC/MS/MS	Tandem mass spectrometry
MOE	Margin Of Exposure
NAA	N-acetylaspartate

NAG	N-acetylglutamate
NAGly	N-acetylglycine
NAS	N-acetylserin
NAT	N-acetylthreonine
NDF	Neutral Detergent Fibre
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
ORFs	Open reading frames
PCR	Polymerase chain reaction
PMEM	Post-market environmental monitoring
PMM	Post-Market Monitoring
RBD	Refined, bleached, and deodorised
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
T-DNA	Transfer-deoxyribonucleic acid

tpt	Triose Phosphate Transporter gene