Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

VKM Bulletin 2026:10

Assessment of new information in the renewal application (notification C/NL/04/02-002) for transgenic carnation event FLO-40644-6 (Florigene®Moonlite™) for import as cut flowers for ornamental use in accordance with article 17 of Directive 2001/18/EC

This assessment is an update to the original VKM health and environmental risk assessment of transgenic carnation event FLO-40644-6 (VKM Report 2015:15).

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Cover photo: VKM Report 2015:15

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Summary

The Norwegian Environment Agency (NEA) has requested the Norwegian Scientific Committee for Food and Environment (VKM) to assess whether new scientific information provided in the renewal application (C/NL/04/02-002) for transgenic carnation event FLO-40644-6 changes VKMs previous conclusions in the risk assessment conducted during the original application (VKM Report 2015:15). Additionally, the NEA has asked VKM to add any comments/enquires regarding the application that should be conveyed by the NEA during the EU scientific hearing, which has a deadline 13. June 2026.

VKM concludes that the updated bioinformatic analyses etc. do not indicate an altered risk that would change the conclusions in VKMs previous risk assessment of transgenic carnation FLO-40644-6.

VKM has no comments/enquires regarding the renewal application to be conveyed by the NEA during the EU scientific hearing.

Sammendrag

Miljødirektoratet har bedt VKM om å vurdere hvorvidt ny vitenskapelig informasjon vedlagt i fornyelsessøknaden (C/NL/04/02-002) for transgen nellik FLO-40644-6 endrer konklusjonene i VKMs risikovurdering i forbindelse med den opprinnelige søknaden (VKM Report 2015:15). I tillegg har Miljødirektoratet bedt VKM om å vurdere evt. innspill til søknaden, som Miljødirektoratet kan fremme i EU-kommisjonens vitenskapelige høringsperiode, som har frist 13. Juni 2026.

VKM konkluderer at ny vitenskapelig informasjon i fornyelsessøknaden ikke påvirker VKMs konklusjoner i den opprinnelige risikovurdering av transgen nellik FLO-40644-6. VKM har ingen kommentarer til søknaden for Miljødirektoratet å spille inn under EUs vitenskapelige høring.

Background

The Norwegian Environment Agency (NEA) has requested VKM to assess whether new scientific information provided in the renewal application (C/NL/04/02-002) for transgenic carnation event FLO-40644-6 changes VKMs previous conclusions in the risk assessment conducted during the original application (VKM Report 2015: 15). Additionally, the NEA has asked VKM to add any comments/enquires regarding the application that should be conveyed by the NEA during the EU scientific hearing, which has a deadline 13. June 2026.

Excerpts of new information in the renewal application C/NL/04/02-002

Information on bioinformatic analysis of flanking sequences

Southern and sequencing analyses of the transgenic carnation event FLO-40644-6 demonstrate that the inserted T-DNA insert is contained at two transgene integration sites (at two loci) (Nakamura et al., 2020). The two loci appear to have been inserted in different linkage groups (chromosomes). Longer flanking genomic DNA sequence of FLO-40644-6 has been obtained since the consent to marketing was provided for FLO-40644-6.

In the renewal application, bioinformatic analysis was conducted using the obtained flanking genomic DNA sequence of the two loci – i.e., 150 bp genomic flanking sequence at the 3' (right border; RB) and 150 bp at the 5' (left border; LB) end of each locus – using two carnation genome databases. The two databases used were the DB carnation database developed by Yagi et al. (2014) and the carnation genome deposited at the NCBI carnation database by Zhang et al. (2022). The DB carnation database was not updated since 2014 and therefore no blastn or tblastx was performed.

Each of the four genomic DNA sequences flanking the T-DNA integration in FLO-40644-6 were subjected to blastn, tblastx and blastx.

- [*BLASTn: A nucleotide homology search for finding similar sequences in nucleotide databases by finding local regions of similarity.*
- *tBLASTx: A nucleotide homology search using both sequences of a query nucleotide sequence from all six possible open reading frames.*
- *BLASTx: An amino acid homology search using translated amino acid sequences from query nucleotide sequence*.]

The finding of from the BLAST searches are presented in the "DB flanking sequence results CNL0402001.pdf" and in the "NCBI flanking sequence results CNL0402001.pdf" files.

Findings

Bioinformatic analysis of flanking sequences:

The blastx search using the DB carnation database resulted in no hits to any query sequence. For NCBI carnation database, the most significant hits for the 4 query sequences using the blastn and tblastx search indicate that locus 1 is inserted in linkage group 6 and locus 2 is inserted in linkage group 1, and the two loci are inserted in different linkage groups. Locus 1 is inserted in scaffold 3618 of the carnation genome, while locus 2 is inserted in scaffold 8. The blastx analysis of the DB carnation database failed to generate any hits, indicating that the two loci are not inserted into any of the ORFs contained in the DB carnation database.

To further investigate in more detail the possible presence of coding regions around the insertion sites a more detailed bioinformatic analysis was conducted. The genes of the carnation genome located in the nearest neighbourhood of the transgenic insertion in scaffold 3618 are Dca34987.1 (putative uncharacterized protein from grape) and Dca34988.1 (putative adenosine deaminase from Arabidopsis). While Dca56429.1 (predicted protein from Silene latifolia), Dca56430.1 (putative transcription factor from castor bean) and Dca56431.1 (putative uncharacterized protein from grape) were identified in the nearest neighbourhood of the insertion of locus 2 in scaffold 8.

Overall, the bioinformatic analysis did not provide evidence of which linkage group the insert might be located in and did not clearly indicate a gene in which the insert might be inserted.

Disruption of endogenous genes

ORFs (open reading frames) were generated from the complete insert sequences including all available genomic flanking sequences in 2016, and these ORFs were used in this renewal application. No new ORFs are generated for the current renewal application. The translated protein sequences of these ORFs across the genomic DNA/T-DNA junctions were re-assessed for homology to toxin and allergen proteins using three allergen databases and cross matching of BLAST outputs against a set of toxin-related protein sequences obtained from the UniProtKB/Swiss-Prot database. The file "*1. ORF analysis CNL0402_001.pdf*" provides details of all ORF generated from locus 1 and locus 2 of FLO-40644-6.

The following three on-line allergen databases used were;

1. The FARRP (Food Allergy Research and Resource Program) allergen protein database (Goodman et al., 2016) located at http://www.allergenonline.org/databasefasta.shtml.
2. The Comprehensive Protein Allergen Resource (van Ree et al., 2021) located at https://comparefasta.comparedatabase.org/
3. The Allermatch database located at https://allermatch.org/database.html.

For toxin homology search, a three-step process was used, based on a blastp analysis of the UNIProtKB/Swissprot database.

Step 1. Generation of a list of toxin related entries. to generate a list of toxin related entries a dataset was generated on January 11, 2026, using the search term toxin in the search panel of the BLAST homepage.

Step 2. Blastp analysis of ORFs. The translated sequences of the ORFs were subjected to a blastp analysis with no search term using the BLAST tool located at https://www.uniprot.org/blast.

Step 3. Cross matching. All significant hits (E-value of less than 1.0) from step 2 were assessed to see if any entries matched the entries obtained in step 1.

Homology of ORF to toxin proteins:

The UNIProtKB/Swissprot database was used to determine if the ORFs had significant homology to toxin proteins in the database. After searching all 24 ORF query sequences, nine ORF query sequences identified in the UniProtKB/Swiss-Prot database where at least one hit had an E-value score of less than 1.0 (Bioinformatic analysis of ORF blastp search results CNL0402001.pdf"). For eight of the ORF query sequences the lowest E-values were all 0.1 or greater and bit scores were all 31.6 or lower. There were no instances of 35% identity across the full sequence of the subject entry in the hit. No significant homologies to toxin-related proteins were identified. Though a hit to a toxin-related protein was identified (*Conus virgo* conotoxin ViKr92 to query sequence L2 6.4), the E-value was 0.26 and so highly unlikely to be biologically significant. Identity was restricted to four amino acids, in the pro-peptide region of the toxin protein.

The only query sequence generating significant homology in the blastp was ORF 4.3. This ORF spans the partial tetracycline sequence in locus 1 (this partial sequence is derived from the transformation vector and does not encode tetracycline resistance) and the genomic DNA flanking the insert at the 3' end. None of the hits identified for this ORF also occurred in the set of 11,060 toxin-related proteins. The specificity to sequence hits from a broad range of species was evidence that the cross matches obtained were the result of commonality of sequence within a broad protein group, rather than to a sequence common to toxin proteins.

Homology of ORF to allergen proteins:

To determine the potential allergenicity, 24 ORF query sequences were assessed using three on-line allergen databases (FAARP, COMPARE and Allermatch databases) using the full FASTA, sliding 80mer window method, and 6/8mer exact match tools. The results of the analysis indicate that none of the putative ORFs share significant homology to allergen proteins in the three up-to-date databases screened. For all ORF query sequences the search results showed no significant homology to allergens in full length searches with all E-values greater than 0.01 (E-scores of less than 0.01 are indicative of homology while only those less than 1e-7 might suggest possible functional similarity). Further, the observed highest bit score was 30.8 suggesting that none of the hits obtained were of biological significance.

Overall, the result of the analyses indicated no biologically significant homology to toxins or allergens (Bioinformatic analysis of ORFs for homology to allergen and toxin proteins CNL0402001.pdf).

Updated bioinformatic analysis of the inserted genes in FLO-40644-6:

Analysis of the sequences of the three newly expressed proteins (CYTB5, F3'5'H and ALS) in FLO-40644-6 using up-to-date protein sequence databases indicated no biologically significant homology to toxins or allergens. The three newly expressed proteins are ubiquitous, well characterized proteins and are not known to be allergens or to possess toxic attributes (Bioinformatic analysis of inserted genes pCGP1470.pdf).

Allergen database searches of the three proteins: In full FASTA alignments, E-values were greater than 0.01 for all query sequences and so are not likely to represent relevant matches (E-scores less than 0.01 are indicative of homology while only those less than 1e-7 might suggest possible functional similarity). No hits were generated from the 80mer sliding window searches for the three databases examined, for any query sequence. In addition to E-score, the Allermatch and FAARP databases generate bit scores for full length searches and the highest bit score for any full length FASTA search was 31.9, indicating that none of the hits obtained were of biological significance. Further, one hit was obtained in the 8mer searches and 37 hits in the 6mer search. As there was no evidence of significant homology to allergens from the full length and sliding window searches, these hits are probably the result of random homology. The analysis of the three newly expressed protein sequences using three allergen protein sequence databases indicated no biologically significant homology to any allergen proteins.

Homology to toxin proteins: Most proteins identified with significant homology to the three query sequences (E-values lower than E^{-20}) were related to the query sequences by function (synthases in the case of ALS, reductases in the case of DFR and Cytochrome P450s in the case of F3'5'H). This was expected, as the inserted genes in the transgenic carnation are ubiquitous and have a well-defined function. None of the inserted genes are known to be toxins and none of the matches were to toxin proteins, including the two dianthin proteins from the organism *Dianthus caryophyllus*. After cross checking, 20 hits generated from the blastp were found in the approximately 11,000 toxin proteins in the UniProtKB/Swiss-Prot database.

The homologies seen in the low E-values are to similarities in protein function and not to motifs specifically related to toxicity. For the F’3’5’H query sequence, all hits were to cytochrome P450 type proteins; the super-family to which flavonoid 3' 5' hydroxylase enzyme belongs. Further, the toxin-related proteins identified by the 15 matches for the F’3’5’H query sequence were enzymes involved in toxin biosynthesis pathways or involved in carcinogen activation. For all hits, identity scores were 22-30% and suggested a minimum identity score of 35% for considering meaningful homology.

Exact amino acid homologies were restricted to just 3-9 amino acids. For the F’3’5’H query sequence, nine of the 15 matches contained the longest continuous sequence homology to parts of the sequence PERFLS within the F’3’5’H protein. For the DFR query sequence, longest amino acid homologies in all 5 matches were to parts of the sequence TGAAGFIGSWLV within the DFR protein. The specificity to these sequences in hits from a broad range of species is evidence that the cross matches obtained were the result of commonality of sequence within a broad protein group, rather than to a sequence common to toxin proteins. Matches 4 and 6 were to the same toxin-related protein but from two different query sequence searches. This is further evidence that the homologies detected are the result of commonality of sequences within a broad family of protein types.

Overall, the analysis indicated no biologically significant homology to toxins or allergens, and all three proteins are ubiquitous, well-characterized proteins which are not known to be allergens or toxins “Bioinformatic analysis of inserted genes pCGP1470.pdf”.

Conclusion

As provided in VKM report 2015:15

"Considering that carnation Moonlite 123.2.38 is not intended for cultivation or use as food or feed, the VKM GMO Panel considers that the comparative analysis of the newly synthesised anthocyanin pigments delphinidin, cyanidin and petunidin in its petals is sufficient for the risk assessment. The reported morphological differences between Moonlite 123.2.38 and its conventional carnation counterpart 123 do not raise safety concerns.

Based on current knowledge, information supplied by the applicant, and considering the intended use which excludes cultivation and use as food and feed, the VKM GMO Panel concludes that Moonlite 123.2.38 is as safe as its conventional counterpart. It is unlikely that the DFR, F3'5'H or ALS proteins, or the delphinidin or cyanidin pigments, will introduce a toxic or allergenic potential in carnation Moonlite 123.2.38.

Likewise, the VKM GMO Panel concludes that carnation Moonlite 123.2.38, based on current knowledge and the intended use as cut ornamental flowers, does not represent an environmental risk in Norway."

28.05.2026 Update, regarding the updated bioinformatics etc. in the renewal application

The VKM GMO panel assessed the provided new information by the applicant, and there is no new evidence in the new information provided by the applicant about – updated bioinformatic analysis of the inserted genes, putative ORFs generated from flanking regions and search for homology to toxin and allergen proteins using updated toxin and allergen databases – that would change VKMs previous conclusions.

References

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Zhang X, Lin S, Peng D, Wu Q, Liao X, Xiang K, Wang Z, Tembrock LR, Bendahmane M, Bao M and Wu Z, 2022. Integrated multi-omic data and analyses reveal the pathways underlying key ornamental traits in carnation flowers. Plant Biotechnology Journal, 20, 1182-1196.

New information C/NL/04/02/001 FLO-40644-6. Document date; February 2. 2026; "*New information CNL0402001.docx"*

Assessment report of the netherlands competent authority in accordance with directive 2001/18/EC. Renewal of notification C/NL/04/02: "*assessment_report_ C-NL-04-02_002.pdf"*