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**DATUM** 18 juli 2022  
**KENMERK** CGM/220718-06  
**ONDERWERP** Advies import en verwerking van gg-koolzaad NS-B50027-4

Geachte mevrouw Heijnen,

Naar aanleiding van een adviesvraag betreffende de vergunning aanvraag voor import en verwerking van genetisch gemodificeerd Koolzaad NS-B50027-4 (EFSA/GMO/NL/2019/160) ingediend door Nuseed, deelt de COGEM u het volgende mee.

**Samenvatting:**

De COGEM is gevraagd te adviseren over de mogelijke milieurisico's van import en verwerking van genetisch gemodificeerd (gg-)koolzaad NS-B50027-4. Deze gg-koolzaad is tolerant voor glufosinaat-ammonium bevattende herbiciden en het zaad heeft een veranderde vetzuursamenstelling.

De moleculaire karakterisering van NS-B50027-4 voldoet aan de eisen van de COGEM. De COGEM merkt op dat niet onderzocht is of de veranderde vetzuursamenstelling van de zaden van invloed is op de predatoren die de zaden consumeren. NS-B50027-4 lijkt een hogere groeikracht te hebben in de vroege groeifase, de COGEM is mede daarom van oordeel dat verscherpte monitoring nodig is om eventuele effecten bij het morsen van NS-B50027-4 zaad in kaart te brengen.

Koolzaadpopulaties komen in Nederland voor op plaatsen waar teelt, transport en overslag van koolzaad plaatsvindt. De COGEM kan op voorhand niet uitsluiten dat er als gevolg van 'gene flow' op termijn 'stapeling' van transgene eigenschappen in koolzaadplanten kan optreden. Mogelijk zou hierdoor een combinatie van transgene eigenschappen kunnen ontstaan waarvan niet van te voren beoordeeld kan worden of zij een schadelijk milieueffect met zich mee zouden kunnen brengen. Op basis hiervan acht de COGEM het noodzakelijk dat er bij gg-koolzaad verplicht gemonitord wordt op locaties waar de kans het grootst is wilde gg-koolzaadpopulaties als gevolg van gemorst zaad aan te treffen, zoals langs transportroutes en bij overslagstations. Tevens zou er aandacht besteed moeten worden aan de introductie van gg-koolzaadzaden via vogelvoer. De COGEM is van mening dat het monitoringsplan hiermee moet worden uitgebreid alvorens de vergunning voor import en verwerking van NS-B50027-4 kan worden afgegeven.



De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

Prof. dr. ing. Sybe Schaap  
Voorzitter COGEM

c.c.

- Drs. Y de Keulenaar, Hoofd Bureau ggo
- Ministerie van IenW, Directie Omgevingsveiligheid en milieurisico's, DG Milieu en Internationaal
- Ing. M.A.C. Möllers, Food-Feed loket

## Import and processing of genetically modified oilseed rape NS-B50027-4 with an altered fatty acid profile

### COGEM advice CGM/220718-06

- The present application (EFSA/GMO/NL/2019/160) concerns the authorisation for import of genetically modified (GM) oilseed rape NS-B50027-4;
- Oilseed rape NS-B50027-4 was produced by *Agrobacterium tumefaciens* mediated transformation. It expresses the *pat* gen, conferring tolerance to glufosinate-ammonium containing herbicides. Additionally, NS-B50027-4 expresses seven transgenes which play a role in the synthesis of omega-3 long-chain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA);
- Feral oilseed rape populations occur across the Netherlands, with a small number of plants (25 or less) per location, along distribution routes and handling areas as a result of spillage of oilseed rape seeds during transport and transshipment;
- Oilseed rape can hybridise with *Brassica rapa* which is a common plant along Dutch roadsides. To a lesser extent it can also hybridise with *Brassica juncea* and *Brassica oleracea*;
- Stable incorporation (introgression) of genes from *B. napus* into wild populations of *B. rapa* and *B. napus* may be possible;
- The molecular characterisation of NS-B50027-4 meets the criteria of COGEM;
- The phenotypic and agronomic characteristics of NS-B50027-4 are comparable to conventional oilseed rape, with the exception of a few characteristics, which differed from the conventional counterpart and fell outside the ranges of non-GM reference varieties;
- The higher early plant vigour of NS-B50027-4 might provide an advantage under certain conditions, COGEM is therefore of the opinion that accidental spillage of NS-B50027-4 and potential effects thereof, should be closely monitored;
- Based on the phenotypic and agronomic characterisation of NS-B50027-4 no indications for potential environmental risks were identified;
- Accidental spillage of GM oilseed rape seeds may lead to the establishment of feral GM *B. napus*, which could lead to plants with stacked events, or feral GM *B. rapa* harbouring GM traits like herbicide tolerance;
- COGEM is of the opinion that the monitoring plan for import and processing of GM oilseed rape events should include monitoring along transport routes (including roadsides and railway beddings) and transshipment areas. This is not included in the current monitoring plan of NS-B50027-4;
- COGEM can only advise positively on the application for import and processing for use in food and feed of NS-B50027-4 oilseed rape, if the monitoring plan is elaborated.

## 1. Introduction

The present application (EFSA/GMO/NL/2019/160), filed by Nuseed as represented by Nufarm B.V., concerns the import and processing of genetically modified (GM) oilseed rape (*Brassica napus*) NS-B50027-4 for use in food, feed and other products. In NS-B50027-4, a metabolic pathway was introduced to alter the fatty acid composition of its seeds. Seven genes encoding for five different fatty acid desaturases and two fatty acid elongases were introduced to produce omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), such as docosahexaenoic acid (DHA). In addition, NS-B50027-4 expresses the *pat* gene, which confers tolerance to glufosinate-ammonium containing herbicides.

## 2. Previous COGEM advice

COGEM has previously advised on the import and processing of GM oilseed rape LBFLFK.<sup>1</sup> This event also contains an altered fatty acid composition. Similar to NS-B50027-4, LBFLFK produces LC-PUFAs. In fact, LBFLFK and NS-B50027-4 both contain genes encoding for the same enzymes in the biosynthetic pathway of DHA. The genes encoding these enzymes were however derived from different donor organisms. In both GM events, these genes were expressed via seed-specific promoters. COGEM was of the opinion that import and processing of oilseed rape LBFLFK poses a negligible risk to the environment in the Netherlands.

## 3. Environmental risk assessment

### 3.1 Characteristics of oilseed rape

Oilseed rape (*Brassica napus*) is a member of the *Brassicaceae* family, which also includes *Brassica rapa*, *Brassica juncea*, *Brassica oleracea* (cabbage), *Brassica nigra* (black mustard) and *Brassica carinata* (Ethiopian mustard). The allotetraploid *B. napus* is the result of natural hybridisation between *B. rapa* and *B. oleracea*.<sup>2,3</sup>

*B. napus* reproduces by self-pollination and cross-pollination. It produces large amounts of pollen, which are dispersed by both wind and insects. In fields, the average rate of cross-pollination is 30%. The seeds of *B. napus* develop in a fruit, and are small, light and produced in large quantities.<sup>2,4,5</sup> Oilseed rape can cross-pollinate with its more common wild relative *B. rapa* and to a lesser extent with *B. juncea* and *B. oleracea*.<sup>2,4</sup>

### 3.2 Receiving environment

In the Netherlands, *B. napus* is grown as a crop and its seeds are imported for oil production. *B. napus* is able to form volunteers in disturbed environments near roadsides, railways and handling areas. The spillage of oilseed rape seeds during transport and transshipment has led to the establishment of feral populations, with a small number of plants (25 or less) per location, along distribution routes and handling areas.<sup>6</sup>

Oilseed rape x *B. rapa* hybrid plants have been observed in the Netherlands.<sup>7</sup> Stable incorporation (introgression) of genes from *B. napus* into wild *B. rapa* has been reported in Canada, but has not been documented in the Netherlands.<sup>8</sup> In a survey performed in the Netherlands in 2019 to investigate

the presence of GM oilseed rape along transport routes and locations where transshipment and processing of oilseed rape takes place, no GM oilseed rape was detected.<sup>9,10</sup> Recent investigation has shown that GM oilseed rape seeds can be present in bird feed mixtures.<sup>11,12</sup> This could pose a potential introduction route of GM oilseed rape into the Dutch environment.

**Conclusion:** Wild *B. napus* populations exist in the Netherlands. *B. napus* can hybridise with its wild relative *B. rapa*. Therefore, GM volunteers from spilled seeds can lead to dispersal of genes to wild populations of *B. napus* and *B. rapa*. To date, no GM oilseed rape has been detected in the Netherlands.

### 3.3 Description of the introduced genes, traits and regulatory elements

Oilseed rape NS-B50027-4 was produced by *Agrobacterium rhizogenes* mediated transformation of oilseed rape variant AV Jade with the binary plasmid vector pJP3416\_GA7-ModB. The genetic elements introduced in NS-B50027-4, and a description thereof, are listed in the table below. The list is limited to information on the introduced genes, corresponding traits, and regulatory elements.

Introduced gene	Encoded protein	Trait	Regulatory elements
<i>Lack1-Δ12D</i>	Δ12-desaturase from <i>Lachancea kluyveri</i>	Converts oleic acid to linoleic acid	Promoter of <i>Linum usitatissimum</i> conlinin 2 (PRO_LinusCnl2) <sup>13</sup> , enhancer from <i>Tobacco mosaic virus</i> (TMV) 59 (TMV 5' UTR leader) <sup>14</sup> , and the terminator of <i>L. usitatissimum</i> conlinin 2 (TER_Linus-Cnl2) <sup>13</sup>
<i>Picpa-ω3D</i>	Ω3-/Δ12-desaturase from <i>Pichia pastoris</i>	Converts linoleic acid to α-linolenic acid	Promoter of <i>L. usitatissimum</i> conlinin 2 (PRO_LinusCnl1) <sup>13</sup> , enhancer from TMV 59 (TMV 5' UTR leader) <sup>14</sup> , and the terminator of <i>L. usitatissimum</i> conlinin 1 (TER_Linus-Cnl1) <sup>13</sup>
<i>Micpu-Δ6D</i>	Δ6-desaturase from <i>Micromonas pusilla</i>	Converts α-linolenic acid to stearidonic acid	Promoter of <i>L. usitatissimum</i> conlinin 2 (PRO_LinusCnl2) <sup>13</sup> , enhancer from TMV 59 (TMV 5' UTR leader) <sup>14</sup> , and the terminator of <i>L. usitatissimum</i> conlinin 2 (TER_Linus-Cnl2) <sup>13</sup>
<i>Pyrco-Δ6E</i>	Δ6-elongase from <i>Pyramimonas cordata</i>	Converts stearidonic acid to eicosatetraenoic acid	Promoter of <i>Arabidopsis thaliana</i> fatty acid elongase 1 (PRO_Arath-FAE1) <sup>15</sup> , enhancer from TMV 59 (TMV 5' UTR leader) <sup>14</sup> , and the terminator of <i>Glycine max</i> lectin (TER_Glyma-Lectin) <sup>16,17</sup>

<i>Pyrco-Δ5E</i>	Δ5-elongase from <i>Pyramimonas cordata</i>	Converts eicosapentaenoic acid to docosapentaenoic acid	Promoter of <i>A. thaliana</i> fatty acid elongase 1 (PRO_Arath-FAE1) <sup>15</sup> , enhancer from TMV 59 TMV 5' UTR leader) <sup>14</sup> , and the terminator of <i>G. max</i> lectin (TER_Glyma-Lectin) <sup>16,17</sup>
<i>Pavsa-Δ5D</i>	Δ5-desaturase from <i>Pavlova salina</i>	Converts eicosatetraenoic acid to eicosapentaenoic acid	Promoter of <i>B. napus</i> napin (PRO_Brana-FP1) <sup>18</sup> , enhancer from TMV 59 (TMV 5' UTR leader) <sup>14</sup> , and the terminator of <i>Agrobacterium tumefaciens</i> nopaline synthase (TER_Agrtu-NOS) <sup>19,20,21,22</sup>
<i>Pavsa-Δ4D</i>	Δ4-desaturase from <i>P. salina</i>	Converts docosapentaenoic acid to docosahexaenoic acid	Promoter of <i>L. usitatissimum</i> conlinin 2 (PRO_LinusCnl2) <sup>13</sup> , enhancer from TMV 59 (TMV 5' UTR leader) <sup>14</sup> , and the terminator of <i>L. usitatissimum</i> conlinin 2 (TER_Linus-Cnl2) <sup>13</sup>
<i>pat</i>	Phosphinothricin acetyltransferase from <i>Streptomyces viridochromogenes</i>	Confers tolerance to glyphosate-ammonium containing herbicides	35S Promoter (constitutive) of <i>Cauliflower mosaic virus</i> <sup>23</sup> , and the terminator of <i>A. tumefaciens</i> nopaline synthase (TER_Agrtu-NOS) <sup>19,20,21,22</sup>

### 3.4 Molecular characterisation

GM oilseed rape NS-B50027-4 was characterised with multiple sequencing approaches, including whole-genome sequencing (WGS), PCR-amplicon sequencing and vector-targeted sequencing (to determine the absence of the vector backbone), and Southern blot analysis.

NS-B50027-4 contains two T-DNA inserts. The insertion in chromosome A05 (46,614 bp) consists of the complete T-DNA region. On chromosome A02 (12,110 bp) the T-DNA region is partially inserted. This part contains four of the seven genes which play a role in the fatty acid synthesis: *Micpu-Δ6D*, *Pyrco-Δ5E*, *Pavsa-Δ5D* and *Picpa-ω3D*. No vector backbone sequences were detected in the genome of NS-B50027-4. On chromosome A05, insertion of the T-DNA replaced a 20 bp long fragment, which interrupted the transcription and function of a predicted Pto-Interacting protein 1-like (*pti*) gene. On chromosome A02, insertion of the T-DNA replaced a 15 bp long fragment inside the 3' UTR of a predicted hypothetical protein (*hpp*) gene. Both the *pti* and *hpp* genes are uncharacterized genes from predicted sequences.

COGEM notes that *pti* could potentially be involved in disease resistance, disruption of this gene might therefore have a negative influence on the fitness of the GM plant.<sup>24,25</sup> This is not further investigated by the applicant. The applicant states that, no significant effects on the agronomy traits were observed (see paragraph 3.5) from the interruption of these endogenous genes. The applicant

argues that this is probably because multiple homologous genes are present in the amphidiploid *B. napus* genome.

The expression of the seven genes of the DHA biosynthetic pathway is driven by seed-specific promoters. Expression of these seven genes was confirmed by analysing developing and mature seeds, no expression was detected in other parts of the plant.<sup>26</sup> The *pat* gene is driven by a constitutive promoter, the PAT protein was present in different tissues of the plant.

Furthermore, the entire insert in each chromosome (A02 and A05) together with the insert-junctions were screened for potential newly created open reading frames (ORFs), from stop codon to stop codon. These ORFs were evaluated for potential similarity to known proteins. No sequence similarities with known putative allergens or toxins were detected in these bioinformatics analyses.

The molecular characterisation was conducted according to the criteria previously laid down by COGEM.<sup>27</sup>

<b>Conclusion:</b> The molecular characterisation of oilseed rape NS-B50027-4 is adequate
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### ***3.5 Phenotypic and agronomic characteristics of NS-B50027-4***

The applicant evaluated the phenotypic and agronomic characteristics of oilseed rape NS-B50027-4, including plant biology and morphology, agronomic performance, and common breeding parameters. NS-B50027-4 differed from the conventional counterpart in a number of the examined characteristics (final stand count, lodging, yield, seeds per pod, early stand count, early plant vigour). The applicant states that the significant differences for these parameters can be considered as not biologically relevant as the differences were not consistent for all field trial sites. COGEM notes that the GM event has an 'early plant vigour' ratio of 1.394, compared to the non-GM counterpart. This indicates that the NS-B50027-4 plants grow 39.4% faster in the early growth phase, compared to the non-GM counterpart. This could give NS-B50027-4 an early advantage when competing with other plants and might lead to establishment of the GM-plant when seed is spilled. Whether the higher early plant vigour of NS-B50027-4 impacts the invasiveness of the plant depends on multiple factors, as for example potential trade-offs, local competition and environment. Therefore, COGEM is of the opinion that accidental spillage of NS-B50027-4 seeds and potential effects thereof, should be closely monitored (see paragraph 5).

Additionally, COGEM notes that there are indications that the presence of some fatty acids may alter the attractiveness of seeds to predators<sup>28</sup> and could affect the seed predation rate. If predators would avoid NS-B50027-4 seeds, the number of NS-B50027-4 plants in disturbed areas may increase. The potential effect of the altered fatty acid composition on seed predation has not been investigated by the applicant. COGEM is of the opinion that the applicant should have discussed the likelihood of an altered predation rate and potential consequences thereof. Overall, it is not probable that the change in fatty acid composition of the seeds alone alters the invasiveness of the plant.

In summary, based on the phenotypic and agronomic characterisation there are no indications that NS-B50027-4 oilseed rape poses an environmental risk. COGEM notes that the higher early plant vigour of NS-B50027-4 might provide an advantage under certain conditions, and therefore recommends to closely monitor accidental spillage of NS-B50027-4 and potential effects thereof.

**Conclusion:** There are no indications that NS-B50027-4 oilseed rape poses an environmental risk. Accidental spillage of NS-B50027-4 seeds should be closely monitored, as the higher early plant vigour of NS-B50027-4 might provide an advantage under certain conditions.

#### **4. Food/feed assessment**

This application is submitted under Regulation (EC) 1829/2003, therefore a food/feed assessment is carried out by EFSA and national organisations involved in the assessment of food safety. In the Netherlands, a food and/or feed assessment for Regulation (EC) 1829/2003 applications is carried out by Wageningen Food Safety Research (WFSR). The outcome of the assessment by these other organisations (EFSA, WFSR) was not known when this advice was completed.

#### **5. Post-market environmental monitoring (PMEM)**

The applicant supplied a general surveillance plan as part of the PMEM. On several occasions, COGEM has expressed concerns with regard to the PMEM plan of GM oilseed rape events. Feral oilseed rape populations can arise from GM oilseed rape seeds spilled during transshipment and transport. Additionally, GM oilseed rape seeds can potentially be introduced in the environment via bird feed mixtures, this introduction route should be considered in the PMEM plan.<sup>11,12</sup> It can be recommended to include producers and distributors of seed mixtures in the PMEM plan, and offer a point where adverse effect can be reported.

As mentioned above, the higher early plant vigour of NS-B50027-4 might contribute to the establishment of GM oilseed rape plants under certain circumstances. COGEM therefore requires it necessary to take this property into account and closely monitor for GM oilseed rape at locations where spillage of seeds might occur, taking into account transport routes (including roadsides and railway beddings), transshipment areas and introduction via bird feed mixtures.

If feral GM *B. napus* populations arise, gene flow between different GM oilseed rape events could give rise to stacked GM oilseed rape events with a new combination of GM traits, or feral GM *B. rapa* harbouring GM traits like herbicide tolerance. As it cannot be excluded beforehand that such a newly generated stacked event may have an adverse effect, COGEM is of the opinion that in case of GM oilseed rape an elaborate PMEM plan is needed.



## 6. Overall conclusion

There are no indications that expression of the introduced traits of oilseed rape NS-B50027-4 poses an environmental risk under natural conditions. Import and processing of NS-B50027-4 poses a negligible risk to human health and the European environment. However, accidental spillage of seeds may lead to the establishment of feral GM oilseed rape, including plants with stacked events. The potential impact of the higher early plant vigour of NS-B50027-4 on establishment of GM oilseed rape plants should be taken in to account when monitoring. COGEM is of the opinion that the current monitoring plan should include monitoring along transport routes and transshipment areas. Therefore, COGEM can only advise positively on the application for import and processing for use in food and feed of NS-B50027-4 oilseed rape, if the PMEM plan is elaborated.

## 7. Additional remarks

COGEM notes that, as mentioned previously, the applicant did not investigate or discuss the potential effect of the altered fatty acid composition of the seeds on seed predation. Nor did the applicant discuss the potential consequences thereof. As these aspects are not known, COGEM is of the opinion that an elaborate PMEM plan is needed to carefully monitor the occurrence of potential adverse effects. Although COGEM considers it an omission that the applicant does not discuss these aspects, they do not alter the outcome of the risk assessment.

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