Bird feed and flower seed mixtures

Potential for disseminating genetically modified seeds

CGM 2022-02

ONDERZOEKSRAPPORT

Bird feed and flower seed mixtures

Potential for disseminating genetically modified seeds

January 2022

COGEM Report CGM 2022-02

Greet SMETS, Nicolas DE SCHRIJVER & Patrick L.J. RÜDELSHEIM
PERSEUS BV

Ordering information

COGEM report No CGM 2022-02
E-mail: info@cogem.net
Phone: +31-30-274 2777

Postal address: Netherlands Commission on Genetic Modification (COGEM), P.O. Box 578, 3720 AN Bilthoven, The Netherlands
Internet Download as pdf-file: http://www.cogem.net → publications → research reports

When ordering this report (free of charge), please mention title and number.

Advisory Committee

The authors gratefully acknowledge the members of the advisory committee for the valuable discussions and patience.

Chair: Dr. Ir. Rommie van der Weide (Wageningen University and Research, Agronomy, Crop protection, Biobased economy, COGEM)

Members: Dr. Ir. Jos Wubben (Netherlands Food and Consumer Product Safety Authority (NVWA), Crop protection and phytopathology, COGEM)

Dr. Ir. Guusje Bonnema (Laboratory for plant breeding, Wageningen University & Research, COGEM)

Dr. Tom de Jong (Leiden University, COGEM)

Ir. Margot Spreuwenberg (ILT, Inspectie Leefomgeving en Transport)

Dr. Ir. Marjan Bovers (COGEM secretariat)

Dr. Ir. Eveline Ultee (COGEM secretariat)

Acknowledgements

The authors gratefully acknowledge the members of the Advisory Committee for the valuable discussions. Many thanks also go to producers and retailers who willingly cooperated in this project.

On the cover: Bird feeding house with seed mixture (Lisette van der Knaap, Rotterdam)

Foreword

Flower seed mixtures intended for sowing in gardens, around field margins and public parks are available as well as birdfeed mixtures intended for feeding wild or companion birds. The composition of seed mixtures varies, and may contain seeds that are locally produced but also outside of the EU. It can be imagined that in countries were genetically modified (GM) crops are cultivated, admixture with seeds of GM crops can occur. In Switzerland, seed mixtures intended as bird feed were investigated and were found to contain seeds of genetically modified (GM) oilseed rape plants.

This finding illustrates how seed mixtures could form a potential route for unintended introduction of GM plants in the environment. To obtain a better insight in these potential introduction routes, COGEM commissioned a research project on the potential presence of seeds of GM plants in birdfeed and flower seed mixtures. COGEM aimed to obtain a better estimate of the dissemination routes of GM seeds and improve knowledge on potential environmental exposure by seed mixtures.

The advisory committee of this research project appreciated the interaction with the authors and their quick and efficient approach in this project. The committee is pleased that the researchers took a two-track approach to investigate the potential dissemination of GM plants in seed lots. A theoretical investigation on the composition and origin of the seed mixtures and precautions taken by the industry, was combined with a practical approach in which the potential presence of the GM seeds was analysed with molecular probes targeting frequently occurring sequences in GM crops. The committee is satisfied with the final report, that sheds a light on the possibility of birdfeed and flower seed mixtures forming an introduction route for GM plants in the environment.

Dr. ir. Rommie van der Weide

Chair of the Advisory Committee

Summary

- **Bird feed and seeds for flower/ wildlife patches may lead to introduction of GM plants into the environment. This has implications for the environmental risk assessment, authorisation and management of the GM variants.**

- **In total the components of over 900 mixtures were inventoried, the number of different species in each mix ranging from 3 to 60. For 50 species present in mixtures, GM variants were identified that had advanced to at least confined field trials.**

- **A rationale is presented to identify those species in the seed mixtures with the highest likelihood for GM variants, if present in the seed mixtures, to establish in the Netherlands. While further investigations would be required to evaluate the implications, it provides an indication that these introduction scenarios are realistic.**

- **Molecular testing of a set of bird feed and seeds for flower/ wildlife patches revealed a positive signal in at least some feed batches for aviary and companion birds. Although other factors (e.g., presence of cross-reacting microbial strains) cannot be excluded, this result can indicate the presence of GM variants in the mix. Further confirmation is required as well as more detailed molecular analysis to identify specific GM variant(s) present.**

- **Suggestions for follow-up actions were proposed.**

The evaluation of a placing on the EU market of a genetically modified (GM) crop and its products predominantly considers the main product flows from cultivation and import down to processed consumer products. Unintentional, yet unavoidable dissemination of GM seeds is also considered in the environmental risk assessment (ERA). However, other uses may also result in dissemination of GM seeds in the European environment. Bird feed mixtures and flower seed mixtures intended for sowing in gardens, field edges, roadsides and public parks offer additional scenarios that may lead to dissemination in less controlled environments.

This study aimed at gaining an insight into potential additional dissemination routes of GM plants and further refining the evaluation of possible environmental exposure. The main research questions of the first part of the study were formulated as:

- Is it possible/likely that GM seeds are present in bird feed and flower seed mixes in the Netherlands?
- Can such presence of GM seeds lead to dissemination routes of which environmental risks haven't been evaluated yet?

While both bird feed and flower seed mixtures can lead to introduction in the environment, the different ways in which they are used have implications for compliance with EU GMO legislation and the related risk assessment. Viable seeds in bird feed may end up in the environment via spillage and are therefore unintentional and unavoidable. On the contrary, flower and wildlife patches are sown intentionally.

GMO variants in flower and wildlife patches would be subject to the cultivation conditions imposed by the GMO approval. Post-Market Environmental Monitoring (PMEM) seems relevant for the flower & wildlife patches as well as for the viable bird seed mixes, the latter however possibly reduced as for unavoidable seed losses during commodity imports. Finally, all uses would be subject to GMO traceability and labelling requirements.

In this study, three approaches were followed:
- Establishing an inventory of species used in bird feed or flower seed mixtures and identifying for which of these species GM variants have been documented to be tested in the field and/or with large scale deployment;
- Understanding practices from producers and distributors already addressing the possible presence of such GM variants, and
- Verifying the presence of such variants in a limited number of samples.

Producers and distributors (19 in the flower seed business and 13 for bird feed) were contacted to gain insight on the origin of the seeds and on their approach for managing potential presence of GM variants in their products. In countries where GM crops are not (yet) commercialised and GM field trials are scarce, like most EU Member States, the likelihood of admixing GM seeds with the mixtures is minimal. Seeds for flower mixes are mostly produced locally, whereas seed mixed in bird feed is usually produced abroad, often outside the EU. In these countries GM crops may be produced or tested in field trials, hence the potential of commingling.

Especially some producers of bird feed mixes attach importance to guaranteeing GM-free products, either via testing or via GM-free certificates. Regarding flower seed mixtures the awareness of the producers was less pronounced: tests on the absence of GM seeds are usually not performed and GM-free declarations or certificates are not common, nor seem they be required by customers.

In summary, the likelihood of commingling is largely reduced by:
- Sourcing from production regions with no field trials and cultivation of GM variants;
- Confirming the absence of GM variants by testing and GMO-free certificates, which seems to be well established with bird feed operators; and
- Seed treatment reducing the germination capacity.

Species present in bird feed mixtures and flower seed mixtures were inventoried considering literature, internet searches for seed mixture distributors and producers as well as physical shop visits. Over 900 mixtures, some composed of up to 60 different species, were evaluated to get a broad inventory of the species possibly present in flower and bird feed mixtures. All together 375 flower seed mixtures were surveyed. Among the seed mixtures for feeding birds, those for aviary and companion birds represented the largest variety of mixtures (380 seed mixes).

GM plant species in commerce and/or covered by field trial permits were listed including the authorising countries and were compared with the many species in the seed mixtures. For 50 species present in mixtures, GM variants were identified that had advanced to at least confined field trials. The most abundant species with a GM variant for bird feed were sunflower, wheat, linseed, rapeseed, safflower and sorghum. In the flower seed mixes these were carrot, chicory, clover, lucerne, borage and sunflower.

Based on the different cases, guiding principles (scale of introduction, timing of introduction, region of introduction and identification of preceding issues) were proposed to evaluate the likelihood of adventitious presence of GM variants related to these species. For most of the 50 species identified with GM variants possibly present in bird feed or flower mixes the likelihood to be present in a significant manner remains very low. Also, only a fraction would be able to establish in the environment in the Netherlands.

Fifty batches of different bird seed and flower seed mixes were acquired for molecular verification of sequences specific for some GM variants. It was opted to apply the validated method that is routinely used by industry to trace GM variants in seed lots and which is at the basis for supporting non-GM claims. This method is however designed for the mainstream commodities and related GM variants. With the routinely used molecular probes, at least 80% of the commercial GM variants and nearly 25% of the GM variants covered by field trial permits can be retrieved with certainty. The latter is largely due to the fact that only limited information is available on the inserted sequences and no validated detection method had been published.

Out of the 50 baches analysed, five showed a positive signal for one of the molecular probes. All these batches belonged to the category of feed mixtures for aviary and companion birds and -contrary to the earlier indication that this sector attaches great importance to potential GM presence- hardly any information was available on GMO testing or the use of GMO-free certificates from the producers of the batches from which the positive samples were taken. Still, the numbers are too small to conclude that GM variants are not present in other mixtures. The seeds in the identified bird feed mixtures were able to germinate, hence to potentially establish under suitable conditions. Still, the likelihood that GM variants present in aviary and companion bird feed mixes result in an environmental release is much lower than e.g., for feeding garden and wild birds, since the former are fed mostly indoors and the latter outdoors. Based on the testing approach it is impossible to determine the plant species or the exact nature of the GM variant(s) involved and it can also not be ruled out that the signal is caused by a cross-reacting microbial strain.

Based on this study the authors suggested the following possible actions:

- **Consider identification and quantification of GM variants in positive samples;**
- **Inform and involve the actors of the product chain;**
- **Include the evaluation of dissemination via mixes in the ERA;**
- **Include provisions in the PMEM; and**
- **Evaluate options for enforcement.**

perseus

Samenvatting

- **Vogelvoer en zaden voor bloemenweiden en wildakkers kunnen leiden tot introductie in het milieu van gg-planten. Dit heeft implicaties voor de milieurisicoanalyse, toelating en management van de gg-varianten.**

- **De samenstelling van in totaal meer dan 900 mengsels is nagekeken. Het aantal verschillende soorten in elk mengsel varieerde van 3 tot 60. Voor 50 van die soorten aanwezig in de mengsels werd een gg-variant geïdentificeerd die in zijn ontwikkeling op zijn minst het stadium van veldproef had bereikt.**

- **Er wordt een gedachtegang voorgesteld om die soorten in een zaadmengsel te identificeren waarvoor de kans het grootst is dat gg-varianten, indien aanwezig in de zaadmengsels, zich kunnen vestigen in Nederland. Niettegenstaande verder onderzoek nodig is om de implicaties te evalueren, geeft het een indicatie dat deze introductiescenario's realistisch zijn.**

- **Moleculair testen van een set van vogelvoerzaden en bloemenzaad-mengsels bracht een positief signaal aan het licht in ten minste enkele loten voor volière- en gezelschapsvogels. Hoewel andere factoren (bijv. aanwezigheid van kruisreagerende microbiële stammen) niet kunnen worden uitgesloten, kan dit resultaat wijzen op de aanwezigheid van gg-varianten in de mix. Verdere bevestiging is vereist, evenals een meer gedetailleerde moleculaire analyse om de aanwezige specifieke gg-variant(en) te identificeren.**

- **Suggesties voor vervolgacties werden voorgesteld.**

Bij de beoordeling van het op de EU-markt brengen van een genetisch gemodificeerd (gg-)gewas en afgeleide producten, wordt voornamelijk gekeken naar de belangrijkste productstromen van teelt en import tot verwerkte consumptiegoederen. Onbedoelde, maar niet te vermijden verspreiding van gg-zaden wordt ook in de milieurisicobeoordeling meegenomen. Echter, ander gebruik kan ook leiden tot verspreiding van gg-zaden in het Europees milieu. Vogelvoermengsels en bloemenzaadmengsels bedoeld om te worden uitgezaaid in tuinen, akkerranden, bermen en openbare parken, leveren bijkomende scenario’s waarbij verspreiding in minder gecontroleerde milieus kan plaatsvinden.

Deze studie had tot doel inzicht te verwerven in de mogelijke, bijkomende verspreidingsroutes van gg-planten en de verdere verfijning van de beoordeling van mogelijke milieublootstelling. De belangrijkste onderzoeksvragen van het eerste gedeelte van de studie waren:

- Is het mogelijk/waarschijnlijk dat gg-zaden aanwezig zijn in vogelvoer en bloemenzaad-mengsels in Nederland?
- Kan die aanwezigheid van gg-zaden leiden tot verspreidingsroutes die op hun beurt kunnen leiden tot milieurisico’s die nog niet werden beoordeeld?

Hoewel zowel vogelvoer als bloemenzaadmengsels kunnen leiden tot introductie in het milieu, heeft het verschil in gebruik implicaties voor de naleving van de EU gg-wetgeving en de bijbehorende risicobeoordeling. Levensvatbare zaden in vogelvoer kunnen in het milieu terechtkomen door morsen en zijn daarom onbedoeld en onvermijdelijk. Bloemenweiden en wildakkers daarentegen worden doelbewust gezaaid.

Ggo's in bloemenweiden en wildakkers zouden onderworpen zijn aan de teeltcondities opgelegd bij de ggo-toelating. Milieumonitoring na het in de handel brengen lijkt relevant voor bloemenweiden en wildakkers, zowel als voor levensvatbare vogelzaadmengsels, hoewel voor deze laatste beperkt tot onvermijdbare zaadverliezen bij import. Ten slotte zou elk gebruik vallen onder de ggo-traceerbaarheids- en etiketteringsvoorschriften.

In deze studie werden drie benaderingen gevolgd:

- Opstellen van een inventaris van soorten in vogelvoer en bloemenzaadmengsels en vaststellen voor welke van deze soorten gg-varianten zijn gedocumenteerd om te worden getest in het veld en/of in grootschalig gebruik;
- Inzicht verkrijgen in de praktijken van producenten en distributeurs die de mogelijke aanwezigheid van dergelijke gg-varianten al aanpakken, en
- Verifiëren van de aanwezigheid van dergelijke varianten in een beperkt aantal monsters.

Producenten en distributeurs (19 in het bloemenzaadbedrijf en 13 voor vogelvoer) werden gecontacteerd om inzicht te verwerven over de herkomst van de zaden en hun aanpak voor het beheren van de potentiële aanwezigheid van gg-varianten in hun producten. In landen waar gg-gewassen (nog) niet gecommercialiseerd zijn en gg-veldproeven schaars, zoals in de meeste EU-lidstaten, is de kans op vermenging met gg-zaden minimaal. Zaden voor bloemenmengsels worden meestal lokaal geproduceerd, terwijl zaden in vogelvoeder gewoonlijk in het buitenland worden geproduceerd, dikwijls buiten de EU. In die landen kunnen gg-gewassen worden geproduceerd of getest in veldproeven, en dus is er kans op vermenging.

Vooral sommige producenten van vogelvoermengsels hechten belang aan het garanderen van GM-vrije producten, ofwel via testen of via gg-vrijcertificaten. Voor bloemenzaadmengsels is het bewustzijn bij de producenten minder uitgesproken: testen op de afwezigheid van gg-zaden worden meestal niet uitgevoerd en gg-vrijverklaringen of -certificaten zijn niet gebruikelijk, en worden ook niet vereist door klanten.

Samenvattend, de kans op vermenging wordt grotendeels verminderd door:

- Inkoop uit productiegebieden zonder veldproeven of teelt van ggo-varianten;
- Bevestiging van de afwezigheid van gg-varianten door middel van testen en gg-vrij-certificaten, wat goed ingeburgerd lijkt te zijn bij operatoren in vogelvoeders; en
- Zaadbehandeling die de kiemkracht vermindert.

Soorten die aanwezig zijn in vogelvoermengsels en bloemenzaadmengsels werden geïnventariseerd, rekening houdend met literatuur, zoekopdrachten op het internet naar distributeurs en producenten van zaadmengsels, en fysieke winkelbezoeken. Meer dan 900 mengsels, sommige samengesteld uit wel 60 verschillende soorten, werden geëvalueerd om een brede inventarisatie te verkrijgen van de soorten die mogelijk aanwezig zijn in mengsels van bloemen en vogelvoer. In totaal zijn 375 bloemzaadmengsels onderzocht. Van de zaadmengsels voor het voederen van vogels vertegenwoordigden die voor volière- en gezelschapsvogels de grootste verscheidenheid aan mengsels (380 zaadmengsels).

Gg-plantensoorten in de handel en/of gedekt door veldproefvergunningen werden opgelijst inclusief de autoriserende landen en werden vergeleken met de vele soorten in de zaadmengsels. Voor 50 soorten die in mengsels aanwezig waren, werden gg-varianten geïdentificeerd die in hun ontwikkeling ten minste waren gevorderd tot veldproeven. De meest voorkomende soorten met een gg-variant voor vogelvoer waren zonnebloem, tarwe, lijnzaad, koolzaad, saffloer en sorghum. In de bloemenzaadmengsels waren dit wortel, cichorei, klaver, luzerne, bernagie en zonnebloem.

Op basis van de verschillende gevallen werden richtinggevende principes (schaal van introductie, timing van introductie, regio van introductie en identificatie van eerdere problemen) voorgesteld om de waarschijnlijkheid van de aanwezigheid van gg-varianten te evalueren. Voor de meeste van de 50 soorten die zijn geïdentificeerd met gg-varianten, mogelijk aanwezig in vogelvoer of bloemmengsels, blijft de kans op aanwezigheid van betekenis zeer laag. Ook zou slechts een fractie zich in Nederland in het milieu kunnen vestigen.

Vijftig loten van vogelzaad- en bloemzaadmengsels werden aangekocht voor moleculaire verificatie van sequenties die specifiek zijn voor sommige gg-varianten. Er is gekozen om de gevalideerde methode toe te passen die door de industrie routinematig wordt gebruikt om gg-varianten in zaadpartijen op te sporen en die aan de basis ligt voor het onderbouwen van niet-ggo-claims. Deze methode is echter ontworpen voor de reguliere grondstoffen en gerelateerde gg-varianten. Met de routinematig gebruikte moleculaire sondes kan met zekerheid ten minste 80% van de commerciële gg-varianten en bijna 25% van de gg-varianten die onder veldproefvergunningen vallen, worden teruggevonden. Dit laatste is grotendeels te wijten aan het feit dat er slechts beperkte informatie beschikbaar is over de ingebrachte sequenties en er geen gevalideerde detectiemethode was gepubliceerd.

Van de 50 geanalyseerde loten vertoonden er vijf een positief signaal voor een van de moleculaire sondes. Allemaal behoren ze tot de categorie voeder voor volière- en gezelschapsvogels en - in tegenstelling tot de eerdere indicatie dat deze sector veel belang hecht aan mogelijke gg-aanwezigheid - was er nauwelijks informatie beschikbaar over ggo-testen of het gebruik van ggo-vrij-certificaten van de producenten van de partijen waarvan de positieve monsters zijn genomen. Niettemin zijn de aantallen te klein om te concluderen dat er geen gg-varianten aanwezig zijn in andere mengsels. De zaden in de geïdentificeerde mengsels van vogelvoer konden ontkiemen en kunnen zich dus mogelijk onder geschikte omstandigheden vestigen. Toch is de kans dat gg-varianten die aanwezig zijn in voedermengsels voor volière- en gezelschapsvogels resulteren in een vrijzetting in het milieu veel kleiner dan b.v. voor het voederen van tuin- en wilde vogels, omdat de eerste meestal binnen en de laatste buiten gevoederd worden. Het is onmogelijk op basis van deze testbenadering om de plantensoort en de exacte aard van de betrokken gg-variant(en) vast te stellen en het kan ook niet worden uitgesloten dat het signaal wordt veroorzaakt door een kruisreagerende microbiële stam.

Op basis van deze studie suggereerden de auteurs de volgende mogelijke acties:

- **Overweeg identificatie en kwantificering van gg-varianten in positieve monsters;**
- **Informeer en betrek de actoren van de productketen;**
- **Neem de verspreiding via mixen op in de milieurisicobeoordeling;**
- **Neem bepalingen op in de milieumonitoring na het in de handel brengen; en**
- **Evalueer mogelijkheden voor handhaving.**

Table of contents

Abbreviations

COGEM	*Commissie Genetische Modificatie* Netherlands Commission on Genetic Modification
EU	European Union
ERA	Environmental risk assessment
ggo	*Genetisch gemodificeerd organisme* GMO
GMFF	Genetically modified food and feed
GM	Genetically modified
GMO	Genetically modified organism
GS	General Surveillance (component of PMEM)
ISAAA	International Service for the Acquisition of Agri-biotech Applications
NAK	*Nederlandse Algemene Keuringsdienst* the Netherlands General Inspection Service
NVG	*Nederlandse Voedingsindustrie Gezelschapsdieren* Dutch pet food trade organisation
PCR	Polymerase chain reaction
PMEM	Post Market Environmental Monitoring
WUR	Wageningen University & Research

1 Introduction

The evaluation of a placing on the EU market of genetically modified (GM) crop and its products predominantly considers the main product flows from cultivation and import down to processed consumer products. Unintentional, yet unavoidable dissemination of GM seeds is also considered in the environmental risk assessment (ERA); e.g., the ERA of import and processing of GM crops in the EU also takes into account potential seed losses during transport and transshipment in harbours, railway stations etc.

However, other uses may also result in dissemination of GM seeds in the European environment. Bird feed mixtures and flower seed mixtures intended for sowing in gardens, field edges, roadsides and public parks offer additional scenarios that may potentially lead to dissemination in less controlled environments. Detection of GM oilseed rape in 24 out of 30 bird feed samples tested in 2017 in Switzerland illustrated the plausibility of such scenario (Schoenenberger and D'Andrea, 2017; 2018; Swissinfo.ch[1]). This finding was further refined with information collected over 3 sampling seasons, indicating that in 40 % of the samples no GM oilseed rape was detected, in 48 % a level below 0,5% of the *Brassica* fraction and in 12 % a level higher than 0,5%. The percentage of contaminating GM-*Brassica* was more frequent when the *Brassica* were found as contaminants and not as components (Frick et al. 2018).

Although the levels were typically low, it could indicate a blind spot in the ERA and potentially trigger regulatory compliance issues.

1.1 Purpose of this study

This study aimed at gaining an insight into potential additional dissemination routes of GM plants and further refining the evaluation of possible environmental exposure. The main research questions of the first part of the study were formulated as:

- Is it possible/likely that GM seeds are present in bird feed and flower seed mixes in the Netherlands?
- Can such presence of GM seeds lead to dissemination routes of which environmental risks haven't been evaluated yet?

1.2 Scope

The increasing interest of the general public in biodiversity made that sales for feed for wild birds and flower seed mixtures increased significantly in the past decades (EPPO, 2007). While the approach in Switzerland focussed on bird feed mixes, this study broadened the scope to other uses, each presenting a specific profile and possible ERA challenges, including:

- **Feed for caged birds**
  People keep birds either for production (poultry), sport (e.g., pigeons) or ornamental purposes. Feed for caged birds, especially when housed outside, may be disseminated. The seeds in these mixtures may retain to some extent their capacity to germinate, hence the potential for establishment. However, given the close proximity to humans, it can be expected that some level of volunteer control may be present.

- **Feed for garden / wild birds**
  In the Netherlands, feeding garden birds is predominantly a winter activity, but year-round feeding is more and more becoming popular. Bird feed is administered as scatter or silo feed, usually in the vicinity of houses or pubic areas, allowing observation of feeding birds. Again, seed germination is not a prerequisite, yet it may also not be guaranteed that establishment is prevented.

[1] https://www.swissinfo.ch/eng/genetically-modified-organisms_gm-plants-in-bird-feed-found-in-non-gmo-switzerland/43739064

- **Seed for flower mixes**
  Seeds for flower strips must germinate to allow growing and flowering. Many mixes will be left to set seed for a subsequent generation. This may lead to dispersal via pollen as well as seed. Private persons as well as public organisations may reserve part of the garden and public spaces for flower strips. Also, farmers are encouraged to sow flower strips around their fields. Next to the beauty, seed mixtures may be intentionally composed of species to attract birds, butterflies or other insects. Sowing wildflower strips is especially promoted to support bees and pollinators of agricultural crops (Mergeay & Adriaens, 2013).

- **Seed for wildlife patches**
  Specific mixes are used by e.g., nature conservation organisations to support wild deer, hare, pheasants etc. Primarily intended to provide shelter and rest for the animals, the sowing of crops fits within the Dutch legislation for nature conservation[2] as a duty of care.
  They are intended to germinate and while flowering is not required, it cannot be excluded. They are typically used in less managed environments aiming to support wildlife.

In this study the focus was on mixtures of seed either for bird feeding or for flower strips, rather than investigating the potential of spreading following the sale of individually packed species.

[2] https://www.navigator.nl/document/752b7d972d4084aa894f4f551844c1a5?ctx=59b9aedb3d9ed45adeb9cb7fb67f5c64

2 Framing the relevance

2.1 Relevance for risk assessment

The EU has established a legal framework to ensure that the development of modern biotechnology, and more specifically of GMOs, takes place in safe conditions. Embedding the precautionary principle, a case-by-case risk assessment should always be carried out prior to a placing on the market (deliberate release) of a GMO and/or of a genetically modified food and feed (GMFF). Over the years different Regulations and Guidelines have been issued providing detailed indications on the process and content of such assessments.

In the ERA[3], the level and routes of environmental exposure to the GM plants must be taken into account. Depending upon the intended uses of a GM plant, such as import, processing, food, feed and/or cultivation, the pathways and levels of exposure of the GM plant to the environment will vary.

In the case where the use of GM plant does not include cultivation in the EU, the problem formulation will consider exposure via:

- the accidental release into the environment of propagules, such as seeds, of the GM plant during transportation and processing potentially leading to sporadic feral GM plants and
- indirect exposure, for example, through manure and faeces from the gastrointestinal tracts mainly of animals fed the GM plant, and/or
- organic plant matter either imported as a fertiliser or soil amendment or derived from other bioproducts of industrial processes.

While these already include important exposure routes, it may not guarantee that exposure via the seed mixes subject to this study would be covered. Accidental, unintentional and possibly unavoidable release during transportation is quite different than e.g., intentional introduction of a flower mix. Also, the receiving environment and its management may be very different. In consequence, in case viable GM seeds are present in seed mixes intended to germinate, it is likely that the ERA submitted for import, processing, and use as food and/or feed has not addressed these additional routes of exposure.

Irrespective of striving for completeness, it is not clear at this point if broadening the ERA would reveal not yet considered environmental issues. Feral populations (conventional or GM) are considered an environmental nuisance in particular circumstances and require management. On the contrary, there seems to be little concern over the environmental impact of volunteers from conventional bird feed and flower mixes, in spite of their deployment today. In fact, the use of conventional flower mixes is further encouraged and no specific environmental issue seems to be associated with it. Whether the presence of GM variants in bird feed or flower mixes may lead to environmental issues needs to be further evaluated. This will largely depend on the particular trait(s) of the GM variant, which e.g., may lead to changes in its capacity to establish and to survive.

While the ERA may require adaptation when taking the different scopes into account, the food/feed safety evaluation will be independent of the origin of the product. Grains, as the raw imported commodity or obtained from crops growing in the EU, are the focus of the safety evaluation. They are the first entry of the food/feed chain process and nutritionally/compositionally most of interest to be included in the comparative safety evaluation (EFSA, 2011[4]). Downstream derived products are included in the assessment and exposure evaluation. As discussed in the next section, the scope of the food/feed safety evaluations for products authorised under the GMFF Regulation has followed this approach.

Of course, this is only relevant for products for which an application has been submitted in the EU. No EU risk assessment would be available for GM plants that may have been commingled in the mixes and for which no submission has been made in the EU.

[3] Guidance on the environmental risk assessment of genetically modified plants (EFSA, 2010)
[4] Guidance for risk assessment of food and feed from GM plants (EFSA, 2011)

2.2 Authorised use

Placing a living GMO on the EU market is subject to an authorisation according to Directive 2001/18/EC on the deliberate release into the environment of GMOs. With no threshold being set, any trace of a non-authorised GMO in a product to be released for planting would be considered illegal. Occasionally, seed lots containing GM events not approved for cultivation have been recalled. Even if a product is approved for import, processing, food and feed use, cultivation or deliberate sowing would not be allowed. At this point only one GM product has been approved and is cultivated in the EU, namely maize event MON-ØØ81Ø-6. Nevertheless, the Netherlands had demanded in accordance of Directive 2001/18/EC, Art 26c a restriction of the geographical scope of all applications; including this existing authorisation. Therefore, introduction of any GM seed for sowing would at this moment be considered illegal in the Netherlands.

Regulation (EC) 1829/2003 on genetically modified food and feed also stipulates that no GMO can be placed on the EU market for food and/or feed use unless it is covered by an authorisation. In order to understand its applicability for the products in this study, one must evaluate the definitions related to feed. In fact, Regulation (EC) 1829/2003 refers to the definition in Regulation (EC) No 178/2002 (General Food Law):

> *'feed' (or 'feedingstuff') means any substance or product, including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals;*

Regulation (EC) No 767/2009 on the placing on the market and use of feed, also refers to the definition in Regulation (EC) No 178/2002, yet further specifies "animals":

> '*food-producing animal' means any animal that is fed, bred or kept for the production of food for human consumption, including animals that are not used for human consumption, but that belong to a species that is normally used for human consumption in the Community;*
>
> *'non-food producing animals' means any animal that is fed, bred or kept but that is not used for human consumption, such as fur animals, pets and animals kept in laboratories, zoos or circuses;*

All of the bird feed mixes, whether intended for food producing (e.g., poultry) or non-food producing animals such as ornamental birds, would be subject to a GMFF authorisation. If a product has been approved for GMFF, the scope normally includes any downstream use of this product or products derived from it.

In other cases, the picture may be more complex. In fact, a flower mix may be intended to attract and support a large diversity of insects, but the maintenance of a flower bed would probably not be considered an act of feeding. Similarly, planting of material to provide shelter that occasionally can serve as food for wildlife is not the same as actively feeding these wildlife birds. Such aspects would be normally covered in the ERA, while taking advantage of information that may be available from the GMFF safety assessment.

Approved GMFF can be found on the Community GMO register[5]. It includes single events and breeding stacks in cotton, maize, oilseed rape, soybean, sugarbeet and swede-rape. The authorised products include depending on the case:

- Foods and food ingredients containing, consisting of, or produced from the GM events
- Feed containing, consisting of, or produced from the GM events
- Products other than food and feed containing or consisting of the GM events for the same uses as the parental species with the exception of cultivation.

Given this broad scope it can be assumed that the additional feed uses such as bird feed are adequately authorised for registered products.

[5] https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm

2.3 Management conditions

Even when authorisations are in place, e.g., related to an authorisation for import, processing, food and feed use of a commodity product, there are different obligations for the use of GM plant material.

2.3.1 Authorisation conditions

An authorisation may specify certain management conditions of use; e.g., an insect resistance management plan may be required to mitigate the possible development of insect resistance. As the use in bird and flower seed mixes present very different applications, the party placing the product on the market may not be able to fulfil these conditions.

2.3.2 Post Market Environmental Monitoring (PMEM)

The GMO Deliberate Release legislation includes an obligation to implement a monitoring plan in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOs as or in products after they have been placed on the market.

In case specific safety issues, where identified during the ERA, the monitoring (case-specific) should be directed at the focal species or the assessment endpoints of concern in receiving environments where effects are most likely to be detected, i.e., where there are high levels of exposure of both the assessment endpoint and the GMO. In absence of such indications, one should still perform PMEM, limited to general surveillance (GS), to detect any unanticipated adverse effect related to the authorised uses. GS is a standard requirement, also for import authorisations for food/feed use excluding cultivation.

In the case of non-viable GM material (e.g., derived products not containing any living GMOs), a PMEM is not required. In the case of imported GM products containing viable propagating material, GS plans should consider that in case substantial loss, spillage and establishment might be possible, appropriate volunteer management systems are implemented to restrict environmental exposure. It is not clear if these potential additional dissemination routes discussed in this project warrant a review of the PMEM approach.

2.3.3 Traceability

Regulation (EC) No 1830/2003 puts in place rules to ensure products containing GMOs and food and animal feed derived from them can be traced at all stages in the production and distribution chain. This traceability (the ability to track GMOs and products produced from GMOs at all stages of the production and distribution chain) is key in providing consumers and the traders with information and safeguards about food/feed derived from GMOs.

The Regulation requires sellers to inform trade buyers in writing that a product contains GMOs (or provide a 'declaration of use' for products intended for food or animal feed) as well to communicate the unique identifiers assigned to each GMO under the Regulation. This information should be provided at every stage in the production and distribution chain and kept for 5 years.

2.3.4 Labelling

Regulation (EC) No 1830/2003 also provides labelling requirements of GM products, food and feed. The words "This product contains genetically modified organisms" or "This product contains genetically modified [name of organism(s)]" must be indicated.

For the labelling requirements a threshold has been set to exempt labelling for traces of authorised GMOs in a proportion no higher than 0,9 % per individual ingredient, provided that these traces are adventitious or technically unavoidable. No threshold is foreseen for non-authorised GMOs (zero-tolerance).

2.4 Summary

In this section, the relevance of the presence of GMOs in bird feed and flower seed mixtures has been introduced. The indicated aspects are not applicable for each type of product as summarised in Table 1, providing a comparison for the different products.

Whereas some bird feed mixes may contain seeds that are viable if they have not been devitalised, seeds in flower and wildlife patch mixes must be able to germinate and flourish. They are sown on purpose; therefore "cultivation" is inherent. On the other hand, they are not considered feed as such, whereas bird seed mixes obviously are.

This differentiation in intended use also means that the risk assessment aspects differ. For non-viable bird feed, no environmental issues are expected. Viable seeds in bird feed may end up in the environment via spillage and therefore inadvertently. Flower and wildlife patches are sown intentionally. Safety aspects of (food and) feed use are most relevant for the bird feed mixes, whereas the consumption and interaction with other species for flower and wildlife patches would be rather seen as an environmental aspect. This is also reflected in the authorisations that would be required for the placing on the market of a GM variant.

GMOs in flower and wildlife patches would be subject to the cultivation conditions imposed by the GMO approval. PMEM seems relevant for the flower & wildlife patches as well as for the viable bird seed mixes, the latter however possibly reduced as for unavoidable losses during import and feeding outdoors.

Finally, all uses would be subject to GMO traceability and labelling requirements.

Table 1 Identification of relevant issues related to dissemination routes through bird feed and flower/wild patch seed mixes of GM variants.

	Bird feed mixes		**Flower & wildlife patch mixes**
	Devitalised	**Untreated**	
Viable	-	☒	☒
Cultivation	-	-	☒
Feed	☒	☒	-
Risk assessment			
Environment	-	(unintentional)	☒
Food/ Feed	☒	☒	(ERA)
Authorisation			
Deliberate release	-	(unintentional)	☒
GMFF	☒	☒	-
Management			
Conditions	-	-	☒
PMEM	-	☒	☒
Traceability	☒	☒	☒
Labelling	☒	☒	☒

3 Species inventory

The study in Switzerland focussed on a single species, namely GM oilseed rape, in bird feed mixes. This choice was justified since several GM oilseed rape products had been cultivated for almost 25 years in non-EU growing regions, most of them can be legally imported in the EU, yet are not authorised in Switzerland, and it is a species with small seeds known to be present in some bird feed mixes. For this study, it was the intention to broaden the scope by including different types of bird feed mixes as well as flower and wild patch seed mixes and the component species.

For bird feed, information was collected on mixes destined to feed wild or garden birds on a feeding table or feeding silo, companion birds, birds in aviaries, poultry and pigeons. Yet, only bird feed mixtures that contain whole seeds were taken into account, since pelleted feed and broken seeds are obviously not able to germinate and to cause any environmental issue. Also, peeled/dehulled seeds may not germinate as in so-called “no grow bird seed” mixtures where the treatment (harshly peeling) is damaging the embryo. In other cases, peeled/dehulled seeds germinate more easily and quicker than the complete seed. The latter seeds were included, the first not.

Regarding flower and wild patch seed mixtures all mixtures for private persons, as well as professionals such as farmers or public organisations were included.

In addition, the scope was broadened by establishing the entire inventory of species in the selected mixes, rather than focussing on a single species.

3.1 Information gathering

Information concerning the composition of seed mixes was searched for in three different ways:

- Literature and other study reports, such as the reports by COGEM (2020), EPPO (2007) and Mauer and Bekker (2015).
- An internet search was conducted for seed mixture distributors and producers. A variety of seed mixtures for both bird feed and flower seeds is for sale online. Most sites provide information on the composition. Species names are given in Dutch and in case of flower seed mixtures often also the scientific binomial name is provided. The search emphasised distributors or brands with sales in the Netherlands.
- A selection of Dutch retailers was visited for tracing additional brands not yet covered by the search for online sales. Chain stores as well as local stores were visited, including AVRI Bloem-en Tuincentrum, Boerenbond - Pets Place, Discus, Garden Center Coppelmans, Intratuin, Jumper and Tuincentrum De Bosrand.

Table 2 Summary of distribution of different seed mixes over identified brands

Brands	Number of mixes			
	Garden bird seed mix	Aviary and companion birds	Poultry and pigeon seed mix	Flower seed mixtures
Internet searches				
Advanta/Limagrain				57
Barenbrug				4
Bijeneducatiecentrum				7
Bijenhof				5
Biodivers				28
Blattner		38		
Boer’n Goed	1	6	4	
Cruydt-Hoeck				17

Brands	Number of mixes			
	Garden bird seed mix	Aviary and companion birds	Poultry and pigeon seed mix	Flower seed mixtures
De Bolderik				35
De Bolster				8
DCM				2
Delinature		51	1	
DLF				2
Esschert Design				2
Herbaseeds				12
Hofman				22
Jan Koenings		16		
Konacorn	2	24	3	
Lord				1
MediGran				43
MR seeds & Mixtures				4
Pelgrum Vink Materialen				55
Pieterpikzonen				7+ 15*
Sluis Garden				18
Ten Have				13
Van Dijke Zaden				11
Vaesen	1	122		
Versele Laga	9	83	78	
Vivara	19			
Vivara Pro				7
Voerdenatuur	17	29	5	
Vogelbescherming	12			
Shop visits				
Beaphar*		11*		
Best Life Green+ (CJ WildBird Foods)	1			
Buzzy Friendly Flowers				13*
Coppelmans	1*			
De Bosrand	1			
Discus, Vonk Diervoeders	2*	6*	2*	
ESVE		2*		
Garvo Alfamix			2	
Horti Tops				21*
Intratuin	1*			12*
Kasper Faunafood		11	3	
Oranjeband zaden				11*
Vitakraft	2*	16*		

* no description of composition available

This overview illustrates the broad scope as intended for the study. While it is impossible to obtain a complete picture of the evolving market, the study aimed to capture a broad range to ensure that the diversity of species used in these mixtures would be mapped. The overview also illustrates that the same producer/ distributor usually may have different bird feed products, yet that these brands are clearly distinct from the flower/ wildlife patch mixes. The latter are clearly more seed business based.

In the next step producers and distributors (19 in the flower seed business and 13 for bird seeds) were contacted for further information regarding the potential presence of GM seeds and the origin of the seed (seed production sites). Also, the Dutch pet food trade organisation ("Nederlandse Voedingsindustrie Gezelschapsdieren", NVG) was contacted. Contacts were made by phone and/or email and the following questions were asked:

- What is the origin of the seeds (country of production)?
- Is a check carried out for impurities (weed seeds)?
- Is there an arrangement with the suppliers to guarantee that no GM seeds are present (e.g., via certification)?
- Are seed mixtures tested for the presence of GM seeds?

An additional question for bird feed seeds:

- Are the seeds still able to germinate / are they treated to prevent germination?

3.2 Bird feeds

3.2.1 Mixtures for feeding wild/garden birds

Internet searches identified 61 seed mixtures for 7 brands of which the composition was disclosed (Table 2). While a large number of webshops were identified, they commercialise mainly the same brands as the ones listed in the table. Shop visits identified an additional 6 brands, some not providing a detailed description. The total amounted to 63 described seed mixtures.

The most common components are:

- *Arachis hypogaea*
- *Avena sativa*
- *Brassica napus*
- *Brassica rapa*
- *Cannabis sativa*
- *Carthamus tinctorius*
- *Fagopyrum esculentum*
- *Guizotia abyssinica*
- *Helianthus annuus*
- *Linum usitatissimum*
- *Panicum miliaceum*
- *Panicum sp.*
- *Phalaris canariensis*
- *Sorghum bicolor*
- *Triticum aestivum*
- *Vigna radiata*

Maize (*Zea mays*) is mostly present in broken kernels or flakes. Mixes with only dehulled and broken seeds were not counted since in so-called "no grow bird seed" mixtures the treatment is damaging the embryo and seeds may not germinate.

3.2.2 Mixtures for feeding aviary and companion birds

A rich variety of seed mixtures is available, each targeting a specific group of birds. The search of webshops of 8 companies/brands resulted in 369 seed mixes for which the ingredients were listed (Table 2). After shop visits, the descriptions of 11 mixes of one brand were added. Numerous species, in many cases tropical, are used in these mixtures sometimes together with pieces of dried fruit. Cereal grains are often broken, flaked or dehulled, especially for smaller birds. Larger birds such as large parrots are fed whole kernels, e.g., maize. Vegetable seeds may be included as well as weed seeds or so-called wild seeds. In the ingredients list of some mixtures weed seeds or wild seeds are found, without further specification. Also, the terminology grass seeds is used without naming the species. Mixes composed of eggfood, extruded feed and pastes were not included.

Shop visits identified 4 additional brands without specifying the composition of the many products.

3.2.3 Mixtures for feeding poultry and pigeons

Poultry are given grain mixtures as extra, but are more generally fed with pelleted feed. The analytical composition of the pellets is often described, but as this type of feed is not of interest to this report, they were not further studied. Pigeon feed consists primarily of grains. Table 2 only lists the grain mixtures. Six brands were found via internet and a further 3 brands during shop visits.

In general bird seeds for all classes are not treated to prevent germination (e.g., by heat), as this would deteriorate the feed quality.

3.2.4 Production areas

The seed mixed in bird feed is usually produced abroad, often outside the EU. In these countries GM crops may be produced or tested in field trials, hence at least the theoretical possibility of commingling.

Often small seeds of tropical species are included in bird feed. Grain crops have their main market in human nutrition. The grains in bird feed are often rejects from human food or from the seed industry (EPPO, 2007). The main production areas for bird feed ingredients according to EPPO (2007) are provided in Table 3.

Table 3 Some species present in bird feeds and their production areas (Source: EPPO, 2007)

Species	Producing countries
Camelina sativa	Europe
Capsicum annuum	Spain
Carthamus tinctorius	Australia, Argentina, Canada, China, Ethiopia, India, Mexico, the USA.
Chenopodium quinoa	South America
Cuminum cyminum	Cyprus and other European countries
Dipsacus sativus	Europe
Fagopyrum esculentum	Europe
Guizotia abyssinica	India, Nepal, Ethiopia, Myanmar, Bangladesh and countries of eastern and central Africa
Helianthus annuus	Argentina, China, France, East African countries (Kenya, Uganda, Tanzania), Hungary, India, Romania, Russian Federation, Spain, Ukraine, the USA.
Linum usitatissimum	The Netherlands
Oryza sativa	Italy and Argentina
Panicum miliaceum	USA, Australia and Argentina
Phalaris canariensis	Canada (75% of world production), the USA, Argentina, Australia, Hungary, Mexico, Greece, Turkey, Spain, Morocco, the Netherlands, England, Uruguay, Thailand

A remark added for *Phalaris canariensis* says: “Most exports take place as bulk, unprocessed seed shipments and, to a lesser extent, as pre-packaged seed mixtures.” (EPPO, 2007). This allows for contaminants to be included, as may be true for other species as well. The fact that Canada, being the main *Ph. canariensis* producer, also cultivates GM oilseed rape and rape seed, justifies special attention to seed cleaning practices before packaging for customers.

3.3 Flower seed mixtures

Online searches for flower seed mixture brands resulted in 22 webshops which listed the composition of their mixtures (Table 2). All together 375 seed mixtures were surveyed. Four more brands were added after visiting garden centres.

The majority of the companies indicated that they produce their seeds or have them produced in the Netherlands, in EU Member States (specifically Belgium, Denmark, Germany), and also in the United Kingdom. Two of the interviewed companies produced exclusively in the Netherlands, whereas two companies confirmed to also import seeds from outside the EU.

None of the companies indicated to test for the presence or absence of GM seeds. A GM-free certification or declaration is seldom requested by customers or retailers. Nevertheless, 4 companies provide such a declaration or claim to be GM-free on their website. One company explained that this can be guaranteed via traceability and auditing. Organic flower seed providers issue a 100% organic certificate. For these the seeds are harvested in nature and multiplied under their own management. Two companies claimed certificates from the “Nederlandse Algemene Keuringsdienst” (NAK), while others indicated that flower seed mixtures are not subject to an inspection obligation.

A seed inventory was made of all mixtures where this information was available. As Advanta/Limagrain had the most diverse set of flower seed mixtures, the full analysis of these is provided as a representative example (Supplementary information: Flower seed mixtures: example Advanta/Limagrain). In total the Advanta/Limagrain mixtures are composed of 440 different species.

The total collection includes mixtures for all kind of soil types and environments, of annuals as well as biennials and perennials, to attract birds and bees, to offer food and shelter (feed plots or wildlife patches) and to simply enjoy the flowers. The latter category also includes the so-called “carnival mixtures” that are composed of species of striking colours, that are in many cases non-native and are not expected to enhance biodiversity in the Netherlands.

Finally, the authors also included mixes that are offered as promotional material. Some companies are specialised in developing personalised packaging and are creative in providing a wide range of products like little seed bags, seed paper, seed balls etc. Other companies/institutions use flower seed bags or vegetable seed bags for educational/promotional purposes. The “Bijeneducatie-centrum” is an example of such an institution where one can buy seed mixes as well and is therefore included in the table above. The composition was not always indicated, but the review of the available descriptions did not reveal a bias for a species not yet identified.

3.4 Summary

In order to get a broad inventory of the species possibly present in flower and bird feed mixtures, the composition of over 900 mixtures were screened.

While collecting the information, some limitations of the approach were identified:

- The exact composition was not always indicated or shared upon request.
- Names were not always exact, either referring to vague common names or group names (such as “grasses” or “wild seeds”).
- Seed mixes differed significantly in the complexity of their composition: the number of species in bird seeds varied from 4 to 25, in flower seeds from 3 to 60. The ratio of the species in the composition was often not indicated.
- The inventory is based on information provided in the public area. The mixtures were not checked for the actual presence or absence of the species indicated, nor was the presence of other species determined. Others have investigated the actual content of wild flower species mixtures determining the species using seed characteristics (Mauer and Bekker, 2015). The authors concluded that some species were missing, while unreported species were present even after cleaning. Of the 15 studied samples, 14 contained extra species. Extra species were presumably mostly field weeds or species added because of the attractive flowers.

Notwithstanding these limitations, the authors are convinced that the large number of mixtures analysed provides a reliable reference for the intended goals of the study.

4 Identifying species with GM variants

Commingling can only occur with species for which GM variants have been developed. While certainly many species have been developed in the lab, in this study the authors focussed on species with GM variants that had been introduced in the environment. This included large scale, commercial deployment as well as confined R&D field trials. Based on the geographical and temporal distribution, it can be assumed that the likelihood of commingling with commercially deployed GM variants is higher than for those only introduced in limited field trials. Yet, these species were included as they provide an indication of how broad the scope can go beyond commercial plantings.

4.1 Information gathering

4.1.1 Commercial cultivation of GM plants

Information on authorisations to culture GM crops was retrieved from the GM Approval Database[6] by the International Service for the Acquisition of Agri-biotech Applications (ISAAA). Also, countries' websites of the competent authorities were visited. The BioTradeStatus.com website[7] by CropLife International provided additional information on the actual commercial status of several GM events.

The species in the seed mixtures were compared with the species authorised for commercial planting and that are actually commercialised. This resulted in a list of species present in certain seed mixtures for which GM events are in commerce.

4.1.2 The GM field trial database

Perseus maintains an up-to-date database on field trials of GM plants worldwide based on publicly available information. Data are retrieved from the websites from national competent authorities and organisations that report on GMO development such as the United States Department of Agriculture, Foreign Agriculture Service[8]. The database contains information on field trials that were either authorised or were applied for. However, it does not guarantee that a particular field trial was actually conducted. Data are available from 2009 onwards, although for the most important countries, like Argentina, Brazil, Canada, the USA and the EU, also earlier data are included.

Again, information in this field trial database was compared with the species listed in the species inventory (final screening performed in October 2021). The result is a list of seed mixture species for which a field trial with a GM event has been applied for somewhere in the world.

4.2 Screening the inventory

Based on the Perseus field trial database and information on commercial GM crop cultivation, the inventory was screened and species for which a GM event has been tested and/or commercialised were identified.

Table 4 indicates species found in seed mixtures for which an authorisation for cultivation has been granted, and/or for which an authorisation for a field trial has been applied for. The relevant countries are indicated as well. While the composition of seed mixtures differs significantly depending on the use, there is a substantial overlap between GM species that can occur in flower and bird seed mixtures.

[6] https://www.isaaa.org/gmapprovaldatabase/default.asp, last accessed on October 15, 2021
[7] http://www.biotradestatus.com/, last accessed on October 15, 2021
[8] https://www.fas.usda.gov/, last accessed on October 15, 2021

Table 4 **Listing of GM variants authorised in the specified countries for commercial release or field trials**[9] **for species which have been identified in seed mixture for flower/ wild life patches and/or bird feeding (**a - feed for garden birds; b - feed for aviary and companion birds; c - feed for poultry and pigeons).

Species identified in seed mixtures			Species with GM variants		
	Used in mixtures for			Authorisation in	
Indicated name	**bird feed**	**flower**	**Name**	**commercial release**	**field trials in**
Agrostis capillaris; A. canina; A. stolonifera		X	*Agrostis sp.*	-	South Korea
Allium cepa	X [a, b]		*Allium cepa*	-	USA
Arachis hypogaea	X [a, b]		*Arachis hypogaea*	-	India, USA
Borago officinalis		X	*Borago officinalis*	-	Canada
Brassica napus	X [a, b, c]	X	*Brassica napus*	Australia, Canada, USA	Australia, Canada, Chile, Germany, Israel, Japan, Lithuania, Spain, Sweden, United Kingdom, USA
Brassica oleracea		X	*Brassica oleracea*	-	India, Taiwan, United Kingdom, USA
Brassica rapa	X [a, b, c]	X	*Brassica rapa*	Canada	Canada, USA
Brassica rapa	X [a, b, c]	X	*Brassica rapa* var. *pekinensis*	-	South Korea
Camelina sativa	X [b]	X	*Camelina sativa*	-	Canada, Sweden, United Kingdom, USA
Capsicum annuum	X [b]		*Capsicum annuum, Capsicum* sp.	China	Israel, South Korea, USA
Carthamus tinctorius	X [a, b, c]	X	*Carthamus tinctorius*	Australia	Argentina, Australia, Canada, Chile, USA
Cichorium intybus	X [a, b]	X	*Cichorium intybus*	USA	Netherlands
Cucumis melo	X [b]		*Cucumis melo*	-	USA
Cucumis sativus	X [b]		*Cucumis sativus*	-	Poland, USA
Cucurbita sp.	X [b]		*Cucurbita* sp.	USA	Chile, Egypt, USA
Daucus carota	X [b]	X	*Daucus carota*	-	Israel, USA
Glycine max	X [b]	X	*Glycine max*	Argentina, Brazil, Canada, Paraguay, Uruguay, USA	Argentina, Brazil, Canada, Chile, China, Colombia, Costa Rica, Cuba, Czech Republic, Germany, Honduras, Israel, Japan, Mexico, Nigeria, Paraguay, Romania, South Africa, Spain, Uruguay, USA

[9] Field trial permit data are complete for the most important countries as of 2001 till today, and all other countries as of 2009 till today.

Species identified in seed mixtures			Species with GM variants		
	Used in mixtures for			Authorisation in	
Indicated name	**bird feed**	**flower**	**Name**	**commercial release**	**field trials in**
Grass seeds (not specified)	X [b]	X	*Agrostis canina*	-	USA
			Agrostis palustris	-	Canada
			Agrostis stolonifera	-	USA
			Festuca arundinacea	-	France, USA
			Lolium multiflorum	-	USA
			Lolium perenne	-	Australia, Canada, Denmark, USA
			Paspalum notatum	-	USA
			Poa pratensis	-	USA
			Poa pratensis x Poa arachnifera	-	USA
Gypsophila paniculata		X	*Gypsophila paniculata*	-	Israel, Kenya
Helianthus annuus	X [a, b, c]	X	*Helianthus annuus*	-	Canada, USA
Hordeum vulgare	X [b, c]	X	*Hordeum vulgare*	-	Canada, Chile, Czech Republic, Denmark, Germany, Hungary, Iceland, Sweden, USA
Iris pseudacorus		X	*Iris* sp.	-	USA
Lactuca sativa	X [b]		*Lactuca sativa*	-	USA
Lens culinaris	X [b]		*Lens culinaris*	-	Canada
Linum usitatissimum	X [b, c]	X	*Linum usitatissimum*	Canada, USA	Australia, Canada, Czech Republic, Poland, Sweden
Lupinus angustifolius		X	*Lupinus angustifolius*		Australia
Medicago sativa		X	*Medicago sativa*	Argentina, Canada, Mexico, USA	Argentina, Canada, Japan, Mexico, USA
Oryza sativa	X [b, c]		*Oryza sativa*	China, Iran, USA	Argentina, Bangladesh, Brazil, Chile, China, Colombia, Costa Rica, Ghana, Honduras, India, Indonesia, Iran, Israel, Japan, South Korea, Nigeria, Pakistan, Philippines, Spain, Taiwan, Uganda, USA
Panicum sp.	X [a, b]		*Panicum virgatum*	-	USA
Papaver somniferum	X [b]	X	*Papaver somniferum*	-	Canada

Species identified in seed mixtures			Species with GM variants		
	Used in mixtures for			Authorisation in	
Indicated name	bird feed	flower	Name	commercial release	field trials in
Phaseolus vulgaris	X [b]		*Phaseolus vulgaris*	Brazil	Brazil, South Korea, Mexico, USA
Pine seeds	X [b]		*Pinus radiata*	-	USA
			Pinus rigida x Pinus taeda	-	USA
			Pinus sp.	-	USA
			Pinus taeda	-	USA
			Pinus taeda; Pinus rigida x Pinus taeda	-	USA
Pisum sativum	X [b, c]	X	*Pisum sativum*	-	Canada, Czech Republic, Germany, United Kingdom, USA
Rosa sp.	X [b]		*Rosa* sp.	Colombia	Colombia, USA
Sinapis alba		X	*Sinapis alba*	-	Canada
Sorghum bicolor	X [b, c]	X	*Sorghum bicolor*	-	Argentina, Australia, India, Kenya, Nigeria, USA
Tagetes sp., *T. erecta*, *T. patula*		X	*Tagetes* sp.	-	USA
Trifolium repens	X [a, b]	X	*Trifolium repens*		USA
Triticum aestivum	X [a, b, c]	X	*Triticum aestivum*	Argentina	Argentina, Australia, Canada, Chile, China, Egypt, Germany, Hungary, India, Israel, Italy, Kazakhstan, Mexico, Pakistan, Paraguay, Spain, Sweden, United Kingdom, Uruguay, USA
Zea mays	X [a, b, c]	X	*Zea mays*	Argentina, Brazil, Canada, China, Colombia, Cuba, Egypt, EU, Honduras, Pakistan, Panama, Paraguay, Philippines, South Africa, Uruguay, USA, Vietnam	Argentina, Australia, Belgium, Brazil, Burkina Faso, Canada, Chile, China, Colombia, Costa Rica, Cuba, Czech Republic, Denmark, Egypt, El Salvador, Ethiopia, France, Germany, Ghana, Honduras, Hungary, India, Indonesia, Israel, Japan, Kenya, Lithuania, Mexico, Mozambique, Netherlands, Nigeria, Pakistan, Panama, Paraguay, Philippines, Poland, Portugal, Romania, Slovakia, South Africa, Spain, Sweden, Tanzania, Uganda, Uruguay, USA, Vietnam

4.2.1 Bird feed mixes

An overview was made where all seed mixtures for garden birds, aviary and companion birds, and poultry and pigeons are listed (not provided). Regarding the species listed in the mixture description, the species with a GM counterpart are indicated for each of them in the Supplementary information: "Bird feed seed mixtures - GM species" (species without a GM variant are not listed). The species that are present in those seed mixtures are presented in Table 5.

Table 5 Ratio of bird seed mixes that include a species with a GM variant expressed as a percentage of the total number (N) of mixes evaluated

Species	Garden birds % N=63	Aviary and companion birds % N=380	Poultry and pigeons % N=96
Allium cepa	3	1	-
Arachis hypogaea	6	6	-
Brassica napus	5	9	32
Brassica rapa	5	31	16
Camelina sativa	-	2	-
Capsicum annuum	-	1	-
Carthamus tinctorius	2	31	87
Cichorium intybus	2	19	-
Cucumis melo	-	1	-
Cucumis sativus	-	1	-
Cucurbita sp.	-	9	-
Daucus carota	-	3	-
Glycine max	-	<1	-
Grass seeds	-	12	-
Helianthus annuus	56	29	48
Hordeum vulgare	-	5	44
Lactuca sativa	-	20	-
Lens culinaris	-	1	-
Linum usitatissimum	6	52	65
Lolium sp.	-	1	-
Oryza sativa	-	20	53
Panicum sp.	24	21	-
Papaver somniferum	-	12	-
Phaseolus vulgaris	-	1	-
Pinus sp.	-	4	-
Pisum sativum	-	4	74
Rosa sp.	-	7	-
Sorghum bicolor	16	18	96
Trifolium sp.	2	4	-
Triticum aestivum	30	19	91
Zea mays	3	8	68

Sunflower seeds (*Helianthus annuus*) appear in more than half of the garden bird seed mixtures followed by wheat seeds (*Triticum aestivum*, 30%). Dehulled sunflower seeds in these mixes are not counted as they are not able to germinate[10]. Maize is often present as broken kernels and is likewise not counted, hence the low figure for maize (3%).

For the aviary and companion birds half of the mixtures contain linseed (*Linum usitatissimum*). Rapeseed (*Brassica rapa*), safflower (*Carthamus tinctorius)* and sunflower (*Helianthus annuus*) are present in a third of the seed mixtures. The figure for *B. rapa* is uncertain and most probably an overestimation. As the scientific name of the plant species is not indicated in the seed lists of

[10] see e.g. https://www.vogelbeschermingshop.nl/premium-no-mess; and https://www.nl.vivara.be/premium-voedermix-puur?gclid=EAIaIQobChMI48rT2P309AIVDs53Ch1nUwRLEAAYASAAEgIMt_D_BwE

the bird seed mixtures, it is not clear which species – *B. rapa* or *B. napus* - is exactly meant by rapeseed ("raapzaad"). From the enquiries with the producers, it seems that "raapzaad" is used for both species. Only when the name oilseed rape ("koolzaad") was indicated, the mixture was counted as containing *B. napus*, otherwise as *B. rapa*. This confusion of tongues is general in international trade of oilseed rape which is confirmed and extensively discussed in Tamis & de Jong (2010).

The major part of poultry/pigeon feed mixtures is intended for pigeons (89 mixes versus 7 for chicken). As already mentioned, chickens are fed pelleted feed, while grain mixtures can be administered supplementary. Safflower (*Carthamus tinctorius*), sorghum or milo (*Sorghum bicolor*) and wheat (*Triticum aestivum)* are dominating the mixtures for poultry and pigeons. *B. rapa* and *B. napus* are only present in pigeon feed, not in poultry feed.

4.2.2 Flower seed mixes

Similar to bird feed mixtures an overview was also made for flower seed mixtures (Supplementary information: "Flower seed mixtures - GM species"; only the species with GM variants are mentioned). The results are summarised in Table 6. As the composition of mixtures was not always described with the scientific names, the "worst-case" assumption was taken to make the calculations: e.g., when the species mustard ("mosterd") was mentioned, it was assumed that all equal *Sinapis alba*, the species for which a GM variant exists.

Table 6 Ratio of flower seed mixes that include a species with a GM variant expressed as a percentage of the total number (N) of mixes evaluated

Species	Flower seed mixtures % N=360
Agrostis canina	1
Agrostis capillaris	9
Agrostis stolonifera	1
Borago officinalis	10
Brassica napus	2
Brassica oleracea	5
Brassica rapa	4
Camelina sativa	<1
Carthamus tinctorius	3
Cichorium intybus	19
Daucus carota	25
Festuca arundinacea	1
Glycine max	<1
Gypsophila paniculata	1
Helianthus annuus	9
Hordeum vulgare	2
Iris pseudacorus	2
Linum ussitatissimum	8
Lolium perenne	3
Lupinus angustifolius	8
Medicago sativa	12
Papaver somniferum	3
Pisum sativum	2
Poa pratensis	6
Sinapis alba	8
Sorghum bicolor	<1
Tagetes sp.	2
Trifolium repens	17
Triticum aestivum	2
Zea mays	1

The most abundant species appeared to be wild carrot (*Daucus carota*), present in a quarter of the described flower seed mixtures. Other common species in descending order are wild chicory (*Cichorium intybus*), clover (*Trifolium repens*), lucerne (*Medicago sativa*), borage (*Borago officinalis*) and sunflower (*Helianthus annuus*). *B. napus* and *B. rapa* are present in only very few mixtures. They appear in wild meadow, wild field and autumn mixtures to support small game like hares, pheasants and partridges. These species are also included in a few bee mixtures.

5 Likelihood of environmental release

Commingling of non-GM seed lots with GM seed may happen in a variety of ways, e.g., by:

- Volunteers of a previous crop,
- Pollen exchange between fields close to each other,
- Carry-over in machinery for sowing, harvesting, seed cleaning and packaging,
- Mixtures in storage facilities, and
- Human error.

It was not the purpose of this study to investigate the mechanisms that may lead to commingling. Rather, based on interactions with producers and distributors, the authors highlighted some practices that may influence the probability of commingling GM variants.

5.1 Commingling in the cultivation region

In Section 4, the authors presented countries where GM events from different species identified in bird feed and flower mixes have been authorised for commercial deployment and/or for field trials. The next step is to determine whether the seeds that are used in the mixes are effectively sourced in a country where GM events of this species are or were grown.
Producers and retailers were asked about the provenance of the seeds. Due to the multitude of species, it was hard to obtain exact data. Most producers indicated that seeds might be harvested worldwide as is also indicated in Table 3, yet with a preference for European countries.

5.1.1 EU

Some companies source oilseed rape from Germany and linseed from the Netherlands. Sunflowers are produced in Europe. Most seeds for flower mixtures are produced in the Netherlands or another EU member state (e.g., Belgium, France, Denmark, Germany). Two companies produce their seeds exclusively in the Netherlands.

Since in most EU Member States no GM crops are commercialised, the likelihood of admixing GM seeds with the mixtures is minimal. The only exceptions are Spain and Portugal where GM maize event MON 810 is grown. However, this is not relevant for flower seed mixes as they do not include maize. While maize may be included in bird feed, this is mostly as broken seeds. Furthermore, MON 810 is allowed for feed use in the EU.

GM field trials have been performed in the EU, but the surface has been declining drastically in the last decades. When verifying the field trials notified in the European GMO register[11] in the last 10 years in the Netherlands, Belgium, Denmark, France and Germany, almost no recent notifications have been made and the indicated species (apples, barley, maize, poplar, potato, sugar beet, tobacco and wheat) do not fit with the scope of the inventory determined in this study.

Inadvertent mixing of GM seeds with bird and flower seed mixtures, as far as produced in the EU, is therefore very unlikely.

5.1.2 Non-EU

Seeds produced in Africa or Asia are mostly tropical seeds such as sorghum and millets or seeds that otherwise do not survive in the Netherlands. With a few exceptions, so far there have been few countries in Africa and Asia in which field trials and/or commercial introduction of the concerned species have occurred.

Of special interest are the seed lots coming from North and South America. These continents are leaders in the commercial production of GM maize, soybean and oilseed rape. Many field trials have been and are being performed. Components of seed mixtures produced in these countries may be inadvertently mixed with their GM counterpart. Another possibility is the admixture of e.g., GM oilseed rape that appears as a volunteer in production fields and is harvested together with the intended crop. Cleaning of the seed lots is not always removing every impurity.

[11] https://webgate.ec.europa.eu/fip/GMO_Registers/GMO_Part_B_Plants.php

Nevertheless, according to the seed producers the number of species from which seed is sourced in North America is limited, even more so from South America. Also, pricing is an important factor: one species may be purchased in several countries depending on the actual market price and availability.

This is illustrated by information on oilseeds for which GM variants have been identified. Fediol statistics[12] indicate that 45% of the rapeseed (*Brassica napus* and *B. rapa*) imported in the Netherlands in 2019, was sourced from within the EU. The rest is imported mainly from Ukraine (FAOstat[13]) and Australia. Canada, the major producer of GM oilseed rape did not export to the Netherlands in 2019. The Fediol data further show that for linseed and for sunflower about 90 % of the imported material was sourced in the EU.

Table 7 Import quantities of species possibly present in bird feed and flower seed mixes, based on Fediol statistics 2019.

Imported in	Species	Import from EU (Tonnes)	Total import (Tonnes)
the Netherlands	Linseed	61,000	72,000
	Rapeseed*	372,000	824,000
	Sunflower	610,000	659,000
Belgium	Linseed	46,000	548,000
	Rapeseed*	658,000	1,940,000
	Sunflower	110,000	116,000

* (*Brassica napus* and *B. rapa*)

Belgium, another important bird feed provider for the Netherlands, imports more rapeseed and linseed, and for both species a significant higher portion is coming from non-EU countries. The Belgian non-EU imports for rapeseed in 2019 are mainly from Ukraine, Australia and Canada[12].

Australia and Canada deploy GM variants of the indicated species. On the other hand, the government of Ukraine does not permit cultivation of GM crops. Nevertheless, there are reports of illegal GM production for certain crops, mainly soybean, rapeseed and maize. However, Ukraine's grain and oilseeds exports are tested prior to exportation (FAS-GAIN, 2020).

While based on the production region, it is possible to evaluate the possibility for commingling during cultivation and grain handling, there are some uncertainties associated with this approach:

- Diverging regulatory approaches may result in incomplete identification of products that might be considered GMOs in the EU. Regulatory differences between USA, Canada and Europe may become even more pronounced e.g., by the introduction of the SECURE rule in the authorisation procedure by APHIS-USDA, USA[14]. The rule focuses on an organism's properties and not on the method used to produce it.
  Regulatory approaches are also evolving for gene edited plants. Whereas in the EU the Decision of the European Court of Justice has indicated that plants modified with new mutation techniques are subject to the GMO legislation, such organisms are in many countries not regarded as GM organisms and are therefore not reported, possibly ending up in international trade. Thus, plant products, that are GM according to EU rules, may be unnoticed.
- The international trade data cover mainstream commodities. However, with the internet providing easy access to an international market, seed mixtures may be purchased on non-EU webshops. While this is theoretically very well possible, in practice retailers are reluctant to sell internationally to private persons because of custom's obligations (phytosanitary certificates, TRACES obligations, etc.)

[12] https://www.fediol.eu/data/Stat%20seeds%202019.pdf last accessed on September 14, 2021
[13] http://www.fao.org/faostat/en/#data trade database, last accessed on September 14, 2021
[14] https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/biotech-rule-revision/secure-rule/secure-reg-changes

5.2 Testing and GMO-free certificates

All interviewed companies producing bird feed mixes (7 producers responded out of 9) attached importance to the GM-free status of the seeds. This is assured by including GMO-free declarations in purchase contracts, or other arrangements (GM-free production areas). Two companies performed GMO-tests themselves on their seeds, one of them specifically on oilseed rape and maize. One company representative also pointed out that the availability of GM-free seed is becoming problematic and may not remain feasible over time. In contrast with the producers, the retailers of bird feed were less informed regarding the GMO provisions.

Concerning impurities and weed seeds, some companies purchased cleaned seed, others are cleaning themselves depending on their position in the chain. In this perspective, the FEDIAF[15] (representing the European pet food industry) "Guide to Good Practice for the Manufacture of Safe Pet Food"[16] is important. The guide was developed by FEDIAF in consultation with the EC and Member States to support compliance with various EU directives and regulations concerning pet food production (Regulation (EC) No 183/2005 on feed hygiene[17] and Regulation (EC) No 1829/2003 on genetically modified food and feed, amongst others).

One of the tests verifies the absence of *Ambrosia* seeds. *Ambrosia* spp. have been added to the list of harmful botanical impurities that are included in Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed[18]. Feed material and compound feed containing unground grains and seeds should contain a maximum of 50 mg of seeds of *Ambrosia* spp. per kg. The presence of *Ambrosia* seeds may indicate a North American origin, which is of interest since it has been shown that seed produced in North America may inadvertently contain GM *Brassica* spp. (Frick et al., 2018).

17 out of 19 contacted flower seed businesses responded in this study. For flower mixes, tests on the absence of GM seeds were usually not performed. GMO-free declarations or certificates were not common, nor were they required by customers. Two companies provided a GMO-free declaration, one assured by traceability and auditing, the other stating that all reasonable steps have been taken to avoid contamination from GMOs or their derivates. Other companies declared on the website to be GMO-free and/or claimed to sell only organic, GMO-free seeds.

Regarding impurities, seed cleaning is performed either internally or externally. One of the companies claimed to receive NAK-certified seed, whereas another representative stated that flower seed mixtures are not subject to an inspection obligation.

5.3 Seed treatment

Another factor is the effect of seed treatment after harvest, especially for bird feed mixtures. To adapt to the specific bird species' needs, larger seeds are mostly broken e.g., in case of maize. Often seeds are peeled for better uptake e.g., in case of sunflower and oats.

Broken and harshly peeled seeds are well known not to germinate. This is sometimes the case in feed mixes for garden and wild birds, to prevent a 'messy' feeding area. With no capacity to germinate, establishment in the environment is prevented.

5.4 Characteristics of the species

The potential environmental impact of a GM species depends on the characteristics of the parent species and the modified traits. The ability to survive and spread in the Netherlands and the potential

[15] www.fediaf.org last accessed on June 2, 2021
[16] https://fediaf.org/images/FEDIAF_Safety_Guide_February_2018_online.pdf last accessed on June 2, 2021
[17] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1–22.
[18] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10–22.

to cross with other species are important features in this respect (COGEM, 2020). Species that are not winter-hardy and have no compatible relatives in the Netherlands will not produce feral populations. On the other hand, species that are native to the Netherlands like many grass species can spread and survive. This also includes species which have been introduced and that have been able to establish.

Based on the classification approach proposed in COGEM (2020), the species for which a GM variant might be commingled with flower and bird feed mixtures were classified (Table 8).

Table 8 Classification of species with GM variants based on the ability to survive and maintain and/or presence of compatible species in the Netherlands

Not able to survive and maintain**		Able to survive and maintain
Without cross-compatible species	**With cross-compatible species**	
Arachis hypogaea *	*Cucumis melo**	*Agrostis canina*
Capsicum annuum *	*Cucumis sativus*	*Agrostis capillaris*
Glycine max	*Cucurbita* sp.	*Agrostis stolonifera*
Lupinus angustifolius	*Hordeum vulgare*	*Allium cepa*
Oryza sativa *	*Lactuca sativa*	*Borago officinalis* *
Panicum virgatum	*Phaseolus vulgaris*	*Brassica napus*
Paspalum notatum *	*Pisum sativum*	*Brassica oleracea*
Sorghum bicolor *	*Tagetes* sp.	*Brassica rapa*
Zea mays	*Triticum aestivum*	*Camelina sativa*
		*Carthamus tinctorius**
		Cichorium intybus
		Daucus carota
		Festuca arundinacea
		*Gypsophila paniculata**
		Helianthus annuus
		Lens culinaris *
		Linum usitatissimum
		Lolium multiflorum
		Lolium perenne
		Medicago sativa
		Papaver somniferum *
		Pinus sp.*
		Poa pratensis
		Rosa sp.*
		Sinapis alba
		Trifolium repens

Source: modified after COGEM, 2020; * indicates species not listed in the COGEM 2020 report
** the occurrence of volunteers is not considered

Species that are not able to survive and maintain will not present a risk to the environment in the Netherlands. At most they can appear as volunteers, but will not establish feral populations and will disappear in the next season. If species with cross-compatible species present in the Netherlands flower, this could result in a GM variant interspecific hybrid. Although theoretically the GM trait(s) could be introgressed in this way into the endemic species, this was not further considered in this study.

On the other hand, species that are able to survive and maintain populations under conditions in the Netherlands deserve special attention. These species are further discussed in Table 9.

In reviewing the specific cases of these different species, it became apparent that the following criteria were guiding the evaluation of the relative potential for the presence of GM in the seed mixes:

1 **Scale of introduction**	The potential for commingling is higher when a GM variant has been introduced on large/commercial scale. Confined field trials usually are limited in number and scale, and are usually subject to stringent conditions. Nevertheless, the possibility that material is misdirected cannot be excluded. This possibility is low if only some research trials are conducted, but increase when additional field trials are required as part of a development project.
2 **Timing of introduction**	If the introduction spans a recent period, the possibility for the presence in actual batches is more realistic than in case the introduction covered a period already several years ago. At the same time, if GM variants have been introduced only recently, then it is less likely to find them compared with GM variants already introduced for a longer period. As a guiding principle, the potential for commingling was reduced in case the last commercial release was more than 10 years ago and the last field trial permit was more than 5 years ago. This needs to be considered in conjunction with the absence of commingling issues in the past.
3 **Region of introduction**	The potential for commingling is higher when the region of production of the seeds for the mixtures coincides with the region where the GM variant has been introduced. In some cases, the production region is clearly separated from the GM variant introduction region. Nevertheless, this criterion requires a conservative approach, since sourcing may change rapidly depending on availability/ costs of material.
4 **Previous issues**	For some species, commingling has already been noticed in other circumstances, illustrating that controls may not have been adequate for discontinuation and/or field trial confinement. Once disseminated, the GM variants may be more difficult to control and may impact cross-breeding species. As a guiding principle, the potential for commingling of a species was increased in case a previous issue had been identified. On the other hand, whenever such cases were discovered, they also triggered strong mitigation measures and tight control in the product chain. Therefore, in case the last issue was more than 5 years ago, and additional controls had been installed, and no further issues were reported, then the potential for commingling was reduced.

The authors realise that -although these criteria have been used in an objective manner- the evaluation presented in Table 9 can be challenged. Therefore, the ratings indicated for the commingling potential should be seen as a relative indication.

5.5 Characteristics of the GMO

The GM trait plays a role as it can provide a selective advantage, increase the survival potential or invasiveness, increase the fitness or affect food and feed safety. Yet, the ERA of the agronomic traits evaluated so far concluded that the introduced traits do not increase the risk for the environment compared with the non-GM parental species. However, in this study the type of introduced traits in potentially commingled events is not *a priori* known and therefore not further addressed.

Table 9 **Rationale (scale of introduction, timing of introduction, region of introduction and identification of preceding issues) and estimation of the relative commingling potential of GM species in flower seed and bird feed mixtures**

Species	Rationale	Commingling potential
Grasses *(Agrostis* sp.*, Festuca arundinacea Lolium multiflorum, Lolium perenne, Poa pratensis)*	Grasses can present an environmental problem. Worldwide, the biggest grass seed producers are New-Zealand, the USA, Canada and North western Europe. However, for the EU the seeds of these species are primarily produced in the Netherlands and other EU Member States (Germany, Denmark and Belgium). A limited number of GM field trials were performed mostly in North America. Nevertheless, in the past an incident occurred with creeping bent grass (*Agrostis stolonifera*) in Oregon, USA, due to outcrossing[19]. Ultimately, the event was approved in 2016, with a restriction that it would never be commercialised or cultivated again. Despite the importance of this event, the authors believe that the chances of encountering this event in lots produced in Europe are extremely low. Incidentally, the impact of this incident has made grass producers aware of the risks of GM admixture. The probability of admixing a GM grass variant in flower or bird seed mixtures is mainly determined by the limited scale of the releases so far and the location of the production areas for the European market are within the EU. Note: grass seeds also appear as an undefined mixture or may be included as so-called “wild seeds”. The type and provenance of these seeds is unknown. This represents an uncertainty.	Very low
Allium cepa	Only a limited number of GM field trials were conducted in the USA. No further indications so far of remaining traces. The Netherlands are world market leader in the production of onion seed.	Extremely low
Borago officinalis	Only one GM field trial was conducted in Canada. No further indications so far of remaining traces.	Extremely low
Brassica sp. (*Brassica napus, Brassica oleracea, Brassica rapa)*	The most notorious species among the GM species able to survive and to form feral populations are *B. napus* and *B. rapa*. GM *B. napus* or oilseed rape is commercialised in Australia, Canada and the USA. Although not commercialised for cultivation in the EU, field trials have been performed in many countries including Germany. Moreover, incidents have been reported in the EU of conventional oilseed rape commingled with GM oilseed rape (de La Hamaide, 2019). This illustrates that the crop is susceptible to inadvertent presence of GM events. Oilseed rape is most often found in bird feed mixtures as compared to flower seed mixtures. There it may be present also as an impurity of canary grass seed (*Phalaris canariensis*). The main producer of canary	High

[19] https://www.aphis.usda.gov/brs/aphisdocs/15_30001p.pdf

Species	Rationale	Commingling potential
	seed (*Phalaris canariensis*) is Canada, a country where also GM oilseed rape and rapeseed is cultivated. Also, other crops may contain *Brassica* impurities (Van Denderen et al., 2010). The risk of commingling these GM crops is relatively high. The likelihood of commingling directly in *Brassica* seed or as an impurity is relatively high. Several GM oilseed rape events have been authorised for cultivation in different territories. Nevertheless, all events of GM oilseed rape currently[20] commercially deployed in Australia, Canada and the USA are authorised for importation, processing, food and feed use in the EU[21], leaving only the environmental aspect as a potential issue. GM *B. oleracea* was only field tested, not commercialised. The risk is therefore relatively low.	
Camelina sativa	GM developments in *Camelina sativa* are relatively recent. Permits for GM field trials have and are being issued, mostly in Canada and some in the UK. The species may become more important in the future; resulting in an increase of the commingling potential.	Very low
Carthamus tinctorius	Safflower for bird feed is produced in Australia and other countries. Two events of *Carthamus tinctorius* have been recently (2018) authorised for cultivation in Australia (ISAAA[6]). As the crop is further introduced, the likelihood of commingling may increase.	Low
Cichorium intybus	Some field trials have been conducted in the EU and a permit has been applied for GM *Cichorium intybus* in the Netherlands in 2003. The field trials were conducted at a limited scale and there have been no further indications so far of remaining traces.	Extremely low
Daucus carota	Field trials have been conducted to a very limited extent. The seeds for seed mixes are produced in Europe.	Extremely low
Helianthus annuus	While field trials of *Helianthus annuus* were performed in Canada and the USA, the seed for seed mixes are traded primarily within the EU (Fediol, 2019[22]).	Very low
Gypsophila paniculata	This species has been field trialled in Israel and Kenya. *Gypsophila* is imported from both Israel and Kenya only as a cut flower.	Extremely low
Lens culinaris	Field trials have been conducted to a very limited extent until 2003 and there have been no further indications so far of remaining traces. Native to the Mediterranean region, Canada and India are large producers. The seeds for seed mixes are produced in Europe.	No

[20] CropLife International: http://www.biotradestatus.com/, last accessed September 10, 2021.
[21] EU Community register of GM food and feed: https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm, last accessed September 10, 2021
[22] https://www.fediol.eu/data/Stat%20seeds%202019.pdf last accessed on June 2, 2021.

Species	Rationale	Commingling potential
Linum usitatissimum	*Linum usitatissimum* for bird feed is produced in the Netherlands according to EPPO (2007) (Table 3), whereas the only GM event was authorised for cultivation in Canada and the USA in the late 1990-ies (ISAAA[6]). Sales were discontinued in Canada in 2001. In 2009 there has been a contamination of Canadian linseed exports, heavily disrupting Canadian export[23]. This illustrated that traces remained in commerce. Since then, there has been a detailed surveillance clearing the linseed production channels.	Very low
Medicago sativa	Three events and their stacks of *Medicago sativa* are authorised for cultivation in Argentina, Canada, Mexico, USA (ISAAA[6]). The species - native to Europe - appears as a component of flower seed mixtures. Seeds are produced in Europe, Australia and USA, but it is not clear where the production for seed mixtures occurs.	Moderate
Papaver somniferum	Field trials have been conducted to a very limited extent in Canada. The seeds for seed mixes are produced in Europe.	Extremely low
Pinus sp.	In bird feed mixtures pine seeds are mentioned ("dennenzaad"; *Pinus* sp.). It is not clear whether this refers to actual pine seeds or pine nuts ("pijnboompitten"). Pine nuts are harvested in several areas of the world from several *Pinus* species. The species that are listed in field trial database are not among them.	No
Rosa sp.	The GM roses are cultivated in Colombia for the cut flower industry and have nothing in common with the rosehips that are used in bird feed.	No
Sinapis alba	A *Sinapis alba* field trial has been conducted in 2020 in Canada. Its seed production for flower seed mixtures takes place in Europe.	Extremely low
Trifolium repens	GM *Trifolium repens* again is only relevant for North America, whereas seeds for the Dutch market are produced in Europe.	Extremely low

[23] https://www.grainscanada.gc.ca/en/industry/policy/protocols/european/

6 Testing for GMO presence

A study conducted in 2017 – 2018 in Switzerland on samples of bird feed focussed on the presence of GM oilseed rape (Schoenenberger and D'Andrea, 2017; 2018; Swissinfo.ch[24]). Unfortunately, there is only scattered information available on the methods and results. Frick *et al.* (2018) indicated:

- A total of 67 samples over 3 seasons;
- Sample size of manually isolated 500 – 1000 *Brassica* seeds (oilseed rape as a component) and at least 50 seeds (oilseed rape as a contaminant);
- Identifying genetic elements p35S, tNOS, *bar*, *pat*, pFMV, *ctp2-cp4-epsps* and event DP-Ø73496-4.

In 60% of the bird feed samples GM oilseed rape variants were detected, most (48% of all samples) being below the Swiss legal threshold of 0,5%. It was concluded that the GM variants had been approved for import and feed use in the EU.

As this study was expected to extend the scope both in type of products (different types of bird feed as well as flower seed mixtures) and species (not only oilseed rape), a different approach had to be developed, not isolating seeds but analysing complex seed mixtures. Furthermore, given the broad range of potential GM events, an adapted testing strategy had to be considered.

As the previous Section confirmed the broad range of potential species for which GM variants have been identified on one hand, yet also indicated that the low likelihood for finding these commingled in the mixtures. Therefore, the interest for performing this analysis was further discussed with the Advisory Committee. In a monitoring study of feral populations (Luijten *et al*., 2019) no trace of GM material could be detected in 160 samples and this was partly explained by the fact that industry had established routes of sourcing non-GM material. Although the likelihood of finding a GM variant was deemed very low, this experimental part of the study was confirmed to be of interest.

6.1 Sample selection

A total of 50 seed mixtures were identified. On the basis of the indicated composition, mixtures were selected that contained at least one or more species for which a GM variant existed. As so far no data had been generated for flower seed mixtures and because in the previous Sections, it was concluded that in general the flower mixtures were less tested for the presence of GMOs compared to the bird seed mixtures, a bigger portion (30) was included. Nevertheless, seeds for bird feed mixtures remained of interest, as they are more often produced outside Europe and therefore potentially more at risk for commingling.

In the selection of mixtures, an attempt was also made to include some mixtures with the species oilseed rape, linseed, creeping bent grass and sunflowers. Some bird feed mixes also contain so-called “wild” or “weed” seeds. Overall the selected mixtures included 34 different species for which a GM variant had been identified.

Table 10 summarises the selection. Care was taken to include a diverse selection including operators that claim to have non-GMO certificates and/or use GMO tests, or not or for which the status is unknown (marked with ? in the table). Similarly information concerning the origin of the seeds was taken into account when different samples were selected. Also, different mixtures for one of the bird feed brands for which no information was present concerning the composition was included as well. For these mixtures it is not known whether species with GM variants are present.

This distribution was chosen to present the diversity rather than targeting a specific practice. The fact that no information is available does not automatically mean that a certain batch is suspected to have a higher commingling risk.

[24] https://www.swissinfo.ch/eng/genetically-modified-organisms_gm-plants-in-bird-feed-found-in-non-gmo-switzerland/43739064

Table 10 Overview of selected mixtures

	Certificate/ test	Location seed production	GM-variants known	Brands	Samples
Flower seeds	?	?	Yes	1	7
	No	?	Yes	2	6
	No	Europe	Yes	6	11
	Yes	Europe	Yes	1	2
	Yes	Worldwide	Yes	2	4
Bird feed mixtures	?	?	?	1	4
	?	?	Yes	2	6
	Yes	Europe	Yes	1	2
	Yes	Worldwide	Yes	3	8
Total				**19**	**50**

A batch of each of the selected seed mixtures was purchased online or in shops without mentioning the purpose of the purchase. The seed bags were kept at room temperature.

References were carefully noted. Upon acquisition, the composition as indicated on the batch of the delivered material was verified with the expected description based on the advertisement or webshop specifications. Where this could be verified, these descriptions matched except for two cases: in both cases one species was not listed on the batch and in one of these another species was included. Most importantly, none of the species that were left out from the batch composition list was a species with a GM variant. Therefore, choosing those seed mixtures did not nullify the selection.

It was not in the scope of this study to determine if seeds of each of the indicated species were actually present in the mixtures. Mauer and Bekker (2015) had already pointed out that the actual mixture may not always coincide with the indicated composition, with some extra species being present while other may not be included. Also, the study in Switzerland found that GM variants were mostly found when oilseed rape was present as a contaminant and not as a component of the feed. While the mixtures were visually inspected, there was no formal determination of species based on seed characteristics.

The only exception was a superficial screening for the presence of *Ambrosia artemisiifolia* seeds. Denderen *et al.* (2010) discussed two studies on the occurrence of alien plants, especially from the genus *Ambrosia*, in batches of seed imported for fodder and birdseeds in the Netherlands. While *Ambrosia* is considered a quarantine species, they revealed its presence in 65% of analysed wild bird feed mixes. Although for this study *Ambrosia* was not a target given that there are no GM variants known, it was nevertheless flagged as an indicator for commingling with material, in particular possibly originating in North America.

None of the batches contained seeds that without any doubt clearly could be determined to be *Ambrosia.* While *Ambrosia* seeds have a distinct shape, once present in mixtures the features may be less pronounced. In the screening the authors therefore included an “undecided” option, indicating that some seeds shapes resembled *Ambrosia* seeds, but could not be unequivocally determined. This was the case for one batch of flower seeds and one batch of bird feed mixtures.

6.2 PCR analysis

After gently shacking the commercial batch to ensure a homogenous distribution, from each of these two samples (100 gr – 1000 gr), A sample and B sample, were taken and transferred to an anonymised translucent plastic bag only bearing an identification code for Perseus. When all samples were collected, all A samples were transferred to SGS Belgium NV in Antwerp for analysis, whereas all anonymised B samples were retained for hand-over for possible follow-up studies.

SGS Belgium NV has a high reputation for independence, integrity and innovation, operating its GMO testing laboratories under strict guidelines and being accredited to ISO/IEC 17025 and ISO

9001:2008. Internationally recognised methods are used in all of their laboratories, having been validated in each location. Participation in proficiency testing for GMO events is completed annually and they have an International Seed Testing Association (ISTA) accredited laboratory for GMO testing. They routinely offer PCR-based tests to confirm the absence or presence of GM plant material in samples of seed or plant tissue, as well as in processed food samples and complex mixtures. Their approach was selected as it represents what an operator may use at this moment if they wish to test for the presence/absence of GM variants.

The test is PCR-based and the kit used was the "foodproof® GMO Screening Kit, 4 Target" (R302 17) from BIOTECON Diagnostics GmbH, Germany[25]. This product was designed to detect GMOs qualitatively in food and animal feed, including plants and seeds, and is in conformity with the ISO 21569 requirements on qualitative detection of GMOs and derived products by analysing the nucleic acids extracted from the sample under study. The qualitative PCR[26] aims at detecting sequences of the 35S-promoter of cauliflower mosaic virus (CaMV), the 3'-untranslated region of the nopaline synthase gene of *Agrobacterium tumefaciens* (NOS terminator), the *bar* resistance gene (phosphinothricin N-acetyltransferase) of the soil bacterium *Streptomyces hygroscopicus* and the FMV-promoter of the figwort mosaic virus. This selection of sequences is in line with Frick *et al.* (2018), although their focus on isolated oilseed rape seeds made some additional checks possible.

From each of the batches, samples (100 - 500 g) were separately grinded and homogenised. DNA was isolated from 200 mg of this homogenate. Positive and negative controls were provided by SGS. The detection limit was said to be 0.01% relative to the amount of plant DNA[27].

Acknowledging that this testing strategy has been designed for commercial events in the main commodities, food and feed as known in Europe, the authors screened the available information for evaluating the extent to which other GM variants may potentially not be identified. In fact, the chosen PCR strategy will only allow detection of GM variants in which the PCR target sequences are present.

For each of the GM variants of species present in at least one of the batches, the Perseus field trial database was checked for the presence of the PCR target sequences. A total of 18588 field trial permits were identified. For most of these (14579) no information was available on the regulatory sequences present in the GM plants. For 3956 permits one or more target sequences were listed and therefore the GM plants are detectable. Fifty-three permits included GM plants that cannot be detected with the chosen PCR strategy.

The same analysis was conducted for all species in the batches for which GM variants are authorised for commercial cultivation whether currently cultivated or not. A total of 139 single events were identified. One hundred eleven of them are detectable, 19 are not. For 9 events the available information was incomplete to decide on the detectability.

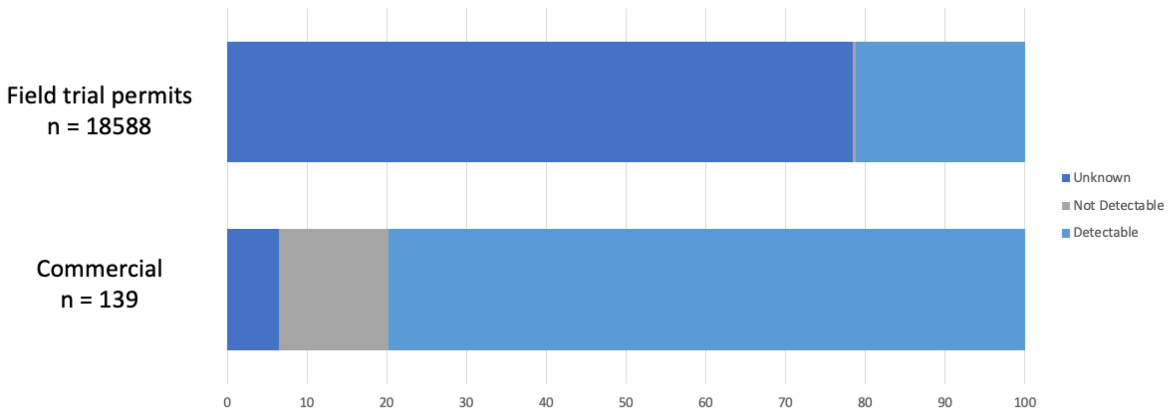

Figure 1 **Relative distribution of permits (field trials and commercial use) for the GM variants which potentially could be present in the selected batches in function of detectability with the PCR strategy** (detectable: one or more PCR target sequences

[25] https://www.bc-diagnostics.com/products/kits/real-time-pcr/gmo/foodproof-gmo-screening-kit-4-targets/
[26] https://gmo-crl.jrc.ec.europa.eu/gmomethods/; ENGL ad hoc working group on "unauthorised GMOs", 2011
[27] Specified as "Limit of Detection (LOD) 0.01% relative GMO content or 1 target copy/µl" on the product sheet.

are present; not detectable: no PCR target sequences are present; unknown: no information on PCR target sequence presence)

No quantitative analysis was conducted as this would not be meaningful for seed mixtures. Sample preparation is performed under sterile conditions. Per run certified reference material is included, as well as a blank sample. The report with the results as received from SGS indicated “positive” or “negative” for each target sequence and for each sample separately.

None of the 30 flower seed batches tested positive for any of the sequences.

Of the 20 bird feed batches, five tested positive for either p35S (1 mixture) or *bar* (4 mixtures) (Table 11). All five belong to the category of feed for aviary and companion birds.

Table 11 Batches with a positive PCR result, with an indication of species mentioned in the batch description for which a GM variant has been identified, with reference to the brand and the communicated production area.

Batch / PCR result		Species indicated in the batch description for which GM variants have been identified	Brand	Production area
ID	**Sequence**			
35	p35S	*Allium cepa, Brassica napus, Brassica rapa, Daucus carota*, Grasses, *Papaver somniferum, Trifolium repens*	X	Not specified
38	*bar*	Composition not specified	Y	Not specified
48	*bar*	*Brassica rapa, Daucus carota*, Grasses, *Helianthus annuus, Lactuca sativa, Linum usitatissimum, Trifolium repens*	Z	Worldwide
49	*bar*	*Brassica rapa, Carthamus tinctorius, Cichorium intybus, Daucus carota,* Grasses, *Helianthus annuus, Lactuca sativa, Linum usitatissimum, Trifolium repens*		
50	*bar*	*Carthamus tinctorius, Cichorium intybus, Daucus carota,* Grasses, *Helianthus annuus, Linum usitatissimum, Oryza sativa, Panicum* sp., *Rosa* sp., *Sorghum bicolor, Triticum aestivum*		

For Brand X, one out of five different mixtures selected for analysis gave a positive result for the p35S sequence. The production area for the components of Brand X had not been specified. Neither was information available on testing or certificates regarding GMOs. As this mixture had been scored on the visual inspection for *Ambrosia* seeds as “undecided”, this could indicate a North-American origin of some of the components.

For Brand Y, one out of four mixtures gave a positive result. Hardly any background information was available on production area or certificates. Also, the composition was not specified on commercial information or on the seed bag. Nevertheless, visual inspection revealed that *Brassica* seed was a major component.

For Brand Z, all (three) tested mixtures gave a positive result for the *bar* sequence. The seeds of this brand are retrieved from different suppliers. No GM-free certificates are obtained, although one of the suppliers was said to test for GM seed presence. Seeds are mostly produced in Western Europe, but can originate from all over the world. *Brassica rapa* was indicated on the composition list of two of the mixtures. A visual screening of the third positive mixture confirmed the presence of *Brassica*-type seeds, although these were not mentioned on the components list (Figure 2).

Figure 2 Presence of *Brassica*-type seeds in sample # 50

The tests performed are routinely used by industry to certify the absence of GM variants in seed products. In case of a positive result, they do not allow to determine the identity of the GM variants (relevant to determine the approval status) or the quantity (relevant to determine the labelling requirements). More detailed molecular analysis will be required to address these, if required. Irrespective, the species listed in the batch composition allow for speculation of potential GM variants.

For the batches with a positive *bar* sequence signal, most species were categorised in the previous Section and indicated with a low likelihood for finding a GM variant. *Linum usitatissimum* was raised as a possible concern given the contamination case that occurred in 2009, however the GM variant that was involved would not be detected with the *bar* screening. *Oryza sativa* is an interesting addition: there have been large scale deployments in China and releases in North America, even leading to contamination of rice imports. Similarly, *Panicum* sp*., Sorghum bicolor* and *Triticum aestivum* could be causing the positive result.

The most likely component showing up as GM variant seems to be *Brassica,* either as a formal component or as an unannounced add-on (e.g., sample 50). Although many *B. napus* events that are commercialised today carry the *bar* gene, they also contain the NOS terminator (see https://www.euginius.eu/; Luijten *et al*., 2019). This is incoherent with the finding that in the analysed samples only the *bar* gene was detected. Further identification would therefore be required to confirm the nature of the possible GM variant.

p35S is a commonly used sequence and can -amongst others- also indicate GM variants of *Brassica* sp.

Finally, a robust determination would also need to consider other sources of the identified sequences. A positive signal from a bacterial contamination or a plant naturally infected with CaMV (Lipp *et al.*, 1999; ENGL ad hoc working group on “unauthorised GMOs”, 2011) might be another possibility. SGS indicated that this was never encountered in their experience, but this may require further investigation given the huge diversity of species and product practices that are typical for bird feed and flower seed mixtures.

6.3 Germination test

The 5 positive seed batches were subjected to a germination test. Seed samples were laid in trays on wetted tissue and incubated at room temperature in daylight (Figure 3).

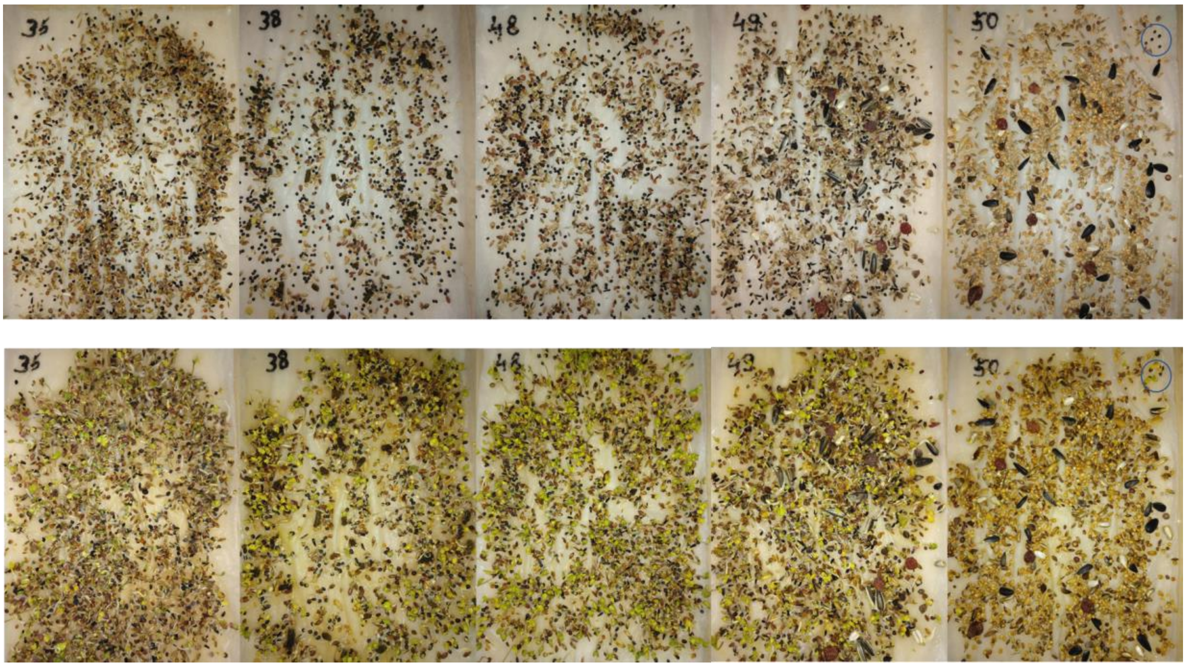

Figure 3 Germination test of PCR positive samples #36, #38, #48, #49 and #50 on day 0 (upper panel) and day 4 (lower panel). The circle in the upper right corner of sample #50 indicates the location of the oilseed rape seeds.

After 96 hours germination was evaluated. All samples included seeds that either had germinated or started to germinate. In all cases, the *Brassica*-type seeds had germinated. Although it is possible that not all seeds/ all species are viable, these results indicated that the mixture as such had not been devitalised. If GM variants would be introduced in the environment, they would be able to germinate under the proper conditions.

After the test all tissues with seeds and seedlings were incinerated.

7 Conclusions

Seed mixes placed on the market to feed birds or to establish plant/flower patches can - in theory - present additional routes for introduction of GMOs in the environment (Table 12). Dissemination in the environment in a targeted manner of even small quantities may, depending on the species, result in establishment of GM variants in the environment. Zünd *et al.* (2019) calculated that a single 300g package of a seed mix used to feed birds in the park is equivalent to a 15-ton container spilling 0.002% of its cargo in transit along a railway line. Although this is an overestimation as it assumes that all the seeds in the bird feed mix are GM and are as viable as the seeds in transit, it marks the importance of these additional scenarios which may not be fully covered in the ERA and the authorisation procedure of GMOs, in contrast to scenarios involving unintended introduction via accidental loss along transportation routes. Table 12 provides an overview of some of the differences between the different utilisations.

Table 12 Differences between specific utilisations on seeds/grain

	Import for processing	Bird feed	Flowers / wildlife patches
Viability	Yes	Yes or No	Yes
Species	Single	Mix	Mix
Impurities	Yes	Yes	Yes
Introduction	Unintentional (transport)	Unintentional (feeding)	Intentional (cultivation)
Environment	Harbours, transportation routes (railways, highways), processing plants	Farms, private, (semi) managed environments such as parks	Farms, private, (semi/un) managed environments
Operators	Grain trade, food & feed processing	Professional (e.g., poultry) Private	Public organisations Professionals Private

These additional routes for exposure of the environment are of particular importance as they lead to dissemination into the environment in a targeted manner which could result -depending on the species- in the establishment of GM plants. Today these routes already exist for non-GM variants and they are not considered to lead to a negative environmental impact. On the contrary, the practices are encouraged. Therefore, the potential environment impact will largely depend on the specific GM trait(s). Irrespective, there may be regulatory restrictions for including GM variants in seed mixtures.

1

Bird feed and seeds for flower/ wildlife patches may lead to introduction of GM plants into the environment. This has implications for the ERA, authorisation and management of the GM variants.

A study carried out in Switzerland in 2017/2018 focussed on GM oilseed rape in bird seed and illustrated the plausibility of such scenario (Frick et al. 2018). Unfortunately, much of the methodological information of this study remains undisclosed.

The current study commissioned by the COGEM broadens the perspective, starting from a wider scope of seed mixtures and species, building a rationale for identifying potential GM variants and targeted testing of selected samples. In addition to feed for garden and wild birds, species present in feed for caged birds, seed for flower mixes and seed for wildlife patches, were surveyed. In total the inventory of over 900 mixtures was reviewed, the number of different species in each mix ranging from 3 to 60.

The limitations of this approach must be recognised (e.g., the species indicated on the components list of the mixtures may not actually be included, whereas other species may be present intentionally or as contaminants). An additional uncertainty comes with the generic indications such as “grasses” and “wild seeds”, where specific species identities are not known or indicated. Nevertheless, the authors are convinced that the large number of mixtures that were reviewed provides a reliable basis for the objectives of the study.

For the surveyed species, it was verified if GM variants had been the subject of an application for a field trial and/or were introduced at commercial scale. This screening resulted in a selection of species for which a viable GM variant exists that may -at least theoretically- end up in seed lots. However, the likelihood of this theoretical possibility is largely reduced by practices indicated by the producers, such as:

- Sourcing from production regions with no field trials nor approved cultivation of GM variants;
- Confirmation of absence of GM variants by testing and the use of GMO-free certificates, which seems to be well established with bird feed operators; and
- Seed treatment reducing the germination capacity (for garden birds).

There is a clear distinction between parties involved and practices for production of mixtures for flower/ wild feeding patches, compared with those for bird feed mixtures.
Finally, also the capacity of the species to establish and maintain populations in the Netherlands influences the potential environmental impact of a release.

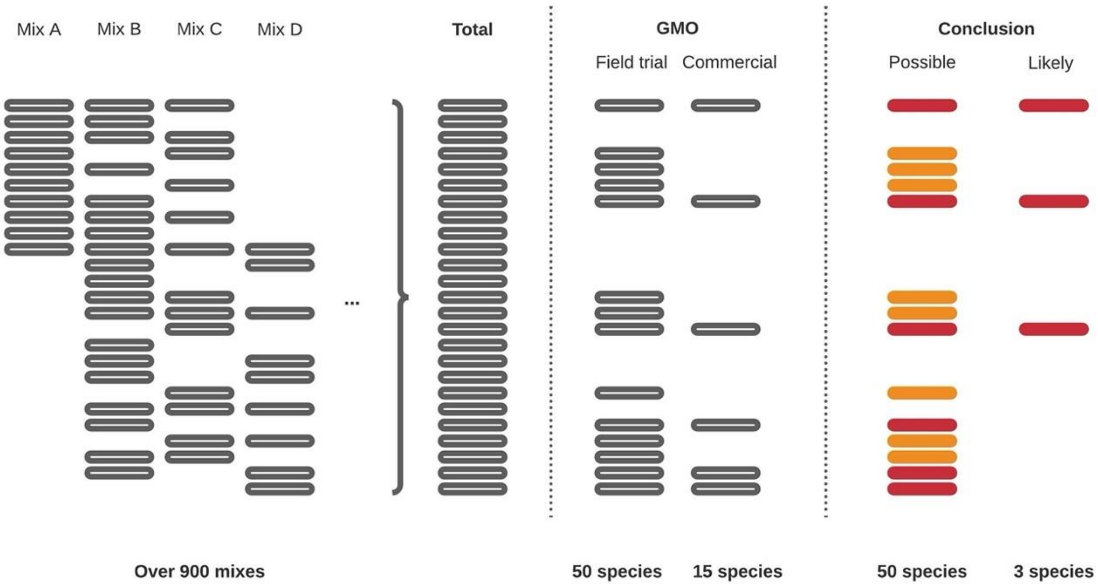

Figure 4 Study approach for the identification and selection of species with GM variants potentially present in seed mixes for bird feed and flower/ wildlife patches in the Netherlands (red indicates species with GM variants in field trials and in commerce; orange species with GM variants in field trials)

Based on the different cases, some guiding principles (scale of introduction, timing of introduction, region of introduction and identification of preceding issues) were proposed to evaluate the likelihood for GM variants to be present. For most of the 50 species identified with GM variants possibly present in bird feed or flower mixes the likelihood to be present in a significant manner is still very low. Also, only a fraction would be able to establish in the environment in the Netherlands.

Given the large-scale deployment in North America and Australia, ***Brassica* spp.** (*Brassica napus, Brassica rapa)* remain the most likely species to be present with GM variants. The confusing indication of different names of the *Brassica* species, makes it difficult to make predictions for any specific lot. In addition to being a prominent component in bird feed mixes, *Brassica* spp. may show up as a contaminant in seed of other species. An example is canary seed (*Phalaris canariensis*) produced in Canada that may contain oilseed rape. This challenge also renders a targeted sampling difficult since contaminants are not communicated.

In addition, ***Medicago sativa*** and ***Carthamus tinctorius*** are highlighted as potential GM variants that can establish in the Netherlands via seed mixes, depending on the controls on their deployment and in view of possible changes in the region of sourcing. Clearly this scope will continue to evolve as GM variants in other species become available and/or their deployment increases.

2

For 50 species present in mixtures, GM variants were identified that had advanced to at least confined field trials.
A rationale is presented to identify those species in the seed mixtures with the highest likelihood for GM variants, if present in the seed mixtures, to establish in the Netherlands. While further investigations would be required to evaluate the implications, it provides an indication that these introduction scenarios are realistic

Based on the information obtained in the market and the identification of species with GM variants, 50 bird seed and flower seed mixes were acquired for molecular verification of sequences specific for some GM variants. It was opted to apply the validated method that is routinely used by industry to trace GM variants in seed lots and which is at the basis for supporting non-GM claims.

This method is however designed for the mainstream commodities and related GM variants. Given the broader scope of species in this study, it was necessary to verify if the methodology would be capable of detecting GM variants in seed mixtures. With the routinely used molecular probes, 80% of the commercial GM variants (single events) can be identified. This percentage is probably higher as possibly some of the commercial events for which it is yet unknown if the method is applicable, may reveal to be detectable. Nearly 25% of the GM variants covered by field trial permits can be retrieved with certainty via the routine detection method. For the majority this is unknown as this uncertainty is largely due to the fact that in most cases only limited information is available on the inserted sequences and no validated detection method had been published. On the other hand, it is likely that many of these GM variants would also include one of the routinely screened sequences and therefore would still be detectable.

The level of uncertainty is expected to increase. Regulatory systems in major production areas (e.g., USA and Canada) are evolving. In particular in relation to applications of gene editing, regulatory discrepancies between approaches in Europe and in other production areas may influence the availability of information on variants that are considered GM in Europe.

With no indication of a particular type of seed mixture that presents a specific potential for commingling, a diverse set of mixtures were sampled and analysed. Out of the 50 samples analysed, five showed a positive signal for one of the molecular probes. Although all belong to the category of feed for aviary and companion birds, the number of samples is too small to conclude that GM variants are not present in other mixtures.

The seeds in the identified bird feed mixtures were able to germinate, hence to potentially establish under suitable conditions depending on the species. Still, the likelihood that GM variants present in aviary and companion bird feed mixes result in an environmental release is much lower than e.g., for feeding garden and wild birds.

Although *Brassica* spp. are at first sight the most likely candidates for which GM variants can be present, based on the testing approach it is impossible to determine the plant species and the exact nature of the GM variant(s) involved. Hence, no conclusion can be drawn on whether the presence indicates an infringement of the EU GM legislation taking into account that most *Brassica* variants have been approved for import and food/feed use and that the exact ratio must be determined to trigger labelling requirements. Furthermore, also other factors like signals caused by non-GM sources of the sequences must be completely ruled out in case of enforcement.

Interestingly, a preceding COGEM study (Luijten et al., 2019) covered the import, distribution and presence of GM *Brassica napus* in the Netherlands. They concluded that no large quantities of GM canola are currently imported into the Netherlands, as also demonstrated by the absence of GM plants in samples of feral populations. This might indicate that the seeds for the mixtures are sourced via different channels than the mainstream commodities.

3

Molecular testing of a set of bird feed and seeds for flower/ wildlife patches revealed a positive signal in at least some feed batches for aviary and companion birds. Although other factors (e.g., presence of cross-reacting microbial strains) cannot be excluded, this result can indicate the presence of GM variants in the mix. Further confirmation is required as well as more detailed molecular analysis to identify specific GM variant(s) present.

Given the complexity of the material and the limited chances for finding commingling in random testing, the authors suggest the following possible actions:

1) Consider identification and quantification of GM variants in positive samples

Within the context of this study, it was the aim to investigate if GM variants can be/are present in bird feed and/or flower seed mixtures and, if so, if these could lead to establishment in the Netherlands. The data presented confirm that these additional dissemination routes for GM variants are realistic.
In order to determine potential compliance issues, a robust analysis of the identity and relative quantity of the GM variants is required, which is beyond the scope of this study.

2) Inform and involve the actors of the product chain

In contrast to major commodity actors that have been confronted with handling GM products since mid-1990-ies, there are still different levels of awareness of GM aspects with actors in the product chains subject to this study. Some parties may be well organised (an example of the pet food sector was provided), whereas others may require further guidance and indications on the applicability of the GM legislation.

It was mentioned that following the 2017 findings in Switzerland, the national authorities contacted bird feed importers to ensure GM seeds do not find their way into the mixes. Interacting with trade organisations, providing information to actors, and sharing experience from commodity trade will strengthen the compliance efforts.

3) Include the evaluation of dissemination via mixes in the ERA

EFSA guidance on the ERA problem formulation of GM plants, already foresees coverage of important potential exposure routes related to import of viable material. For cultivation, this would be a broader range of exposure routes. Still, bird feeding (in particular for garden and wild birds) and flower mixes present additional scenarios. In particular the flower and wildlife patch mixes introduce possibilities for targeted introductions.

While it is possible that this extended ERA reveals no particular environmental risks that have not already been identified, the specificity of the scenarios warrants a focussed evaluation.

4) Include provisions in the PMEM

So far General Surveillance PMEM efforts involves actors in the production and processing chain, again focussing on major commodity trade flow of goods. The scenarios discussed in this study identified other stakeholders, which may not yet be equipped or included to contribute to monitoring and/or serve as an early warning system.

Assuming that producers and distributors of such mixes would be the first to know if a problem occurs, e.g., with difficulties for controlling volunteers, this information would be extremely valuable for assessing how realistic these scenarios are.

It is probably unrealistic to perform in field monitoring, since the location where the seed will be finally used will be very diverse and uncontrollable.

5) Evaluate options for enforcement

In contrast to GM plant varieties, a huge and dynamic variety of mixes is offered. This also presents challenges for enforcement and inspection:

- Random sampling of mixes may not provide a representative picture of the large diversity.
- Targeted interactions with producers and distributors based on the traceability provisions of Regulation (EC) No 1830/2003 can provide an upstream verification of quality systems in place.
- In case observations reveal the possible presence of a GM plant in the environment, investigation should take into account the possibility of an introduction related to one of the scenarios involving seed mixes. This can be a trigger for further verifications upstream of the product chain.

8 Supplementary documents

The following supplementary documents are provided as Excel spreadsheets:

- Bird feed seed mixtures - GM species
  In these tables all seed mixtures for garden birds, aviary and companion birds and poultry and pigeons are listed. For each of them the species with a GM variant are indicated as listed in the mixture description.

- Flower seed mixtures: example Advanta/Limagrain
  Listing all species present in the 57 Advanta/Limagrain flower seed mixtures as an example of the variety of species in flower mixes.

- Flower seed mixtures -GM species
  All flower seed mixtures are listed against the species with a GM variant as listed in the mixture description.

perseus

9 References

COGEM, 2020. Advies inschatting milieurisico's van sporen van niet-toegelaten genetisch gemodificeerde akker- en tuinbouwgewassen in zaaizaad en ander uitgangsmateriaal. CGM/200923-01. 21 pp.

de La Hamaide S., 2019. French, German farmers destroy crops after GMOs found in Bayer seeds. https://www.reuters.com/article/us-france-gmo-bayer-idUSKCN1PV1RG

ENGL ad hoc working group on "unauthorised GMOs", 2011, Overview on the detection, interpretation and reporting on the presence of unauthorised genetically modified materials. Guidance document from the European Network of GMO Laboratories (ENGL). JRC Scientific and Technical Reports. Luxembourg: Publications Office of the European Union. 58 pp.

EPPO, 2007, Pathway analysis: production and processing of small seeds for birds. EPPO Reporting Service no. 06 – 2007. Num. article: 2007/122. 6 pp.

FAS-GAIN, 2020, Biotechnology and Other New Production Technologies Annual, Ukraine, Report Number: UP2020-0057, 23 pp.

Frick, G., Pradervand, N., Boscung, H., 2018. Monitoring bird feed for the presence of undesired and possibly viable seeds harmful for the animals or the environment. Newsletter 2018, IAG section Feed Microscopy, pages 10-11.

Lipp M, Brodmann P, Pietsch K, Pauwels J and Anklam E. 1999. IUPAC Collaborative trial study of a method to detect the presence of genetically modified soybeans and maize dried powder. Journal of AOAC International 82, 923–928

Luijten SH, Seip LA, van Beekvelt C, Fronen B, Prins TW (2019) What is known about the import, distribution and presence of GM oilseed rape (*Brassica napus*) in the Netherlands? COGEM 2020-02 https://cogem.net/publicatie/what-is-known-about-the-import-distribution-and-presence-of-gm-oilseed-rape-brassica-napus-in-the-netherlands/

Mauer, A. and Bekker R., 2015. Inventarisatie samenstelling wilde plantenmengsels. In opdracht van de Nederlandse Voedsel- en Warenautoriteit. 17 pp.

Mergeay J. and Adriaens T., 2013. Afwegingskader voor het gebruik van bloemenzaadmengseld ten bate van bestuivers en biodiversiteit. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2013 (INBO.R.2013.5). Instituut voor Natuur- en Bosonderzoek, Brussel. 34 pp.

Schoenenberger N. and D'Andrea L., 2017. Analysis of (GE) Birdfeed and Sampling of Hotspots of (GE) Oilseed Rape. 17 pp. Unpubl. report for the FOEN (Swiss Federal office for environment).

Schoenenberger N. and D'Andrea L., 2018. Identifikation und Analyse von Vogelfutter-Hotspots in der Schweiz. 5 pp. Unpubl. report for the FOEN (Swiss Federal office for environment).

Tamis W.L.M. and de Jong T.J., 2010, Transport chains and seed spillage of potential GM crops with wild relatives in the Netherlands. COGEM Report CGM 2010-02. 64 pp.

Van Denderen, P.D., Tamis, W.L.M. and van Valkenburg, J.L.C.H., 2010, Risico's van introductie van exotische plantensoorten, in het bijzonder uit het geslacht *Ambrosia*, via import van zaden voor met name veevoer en vogelvoer, Gorteria 34(3), p.65-85.

Zünd, J., Wüst Saucy, A.G., Züghart, W. and Bühler, Ch (2019) Monitoring of Spontaneous Populations of Genetically Modified Plant Species in the Environment - Experiences and Recommendations for the Design of a Monitoring Programme. Report prepared for Joint EPA ENCA Interest Group on Genetically Modified Organisms (IG GMO) 81 pp. https://www.bafu.admin.ch/dam/bafu/en/dokumente/biotechnologie/fachinfo-daten/2019-technical-report-monitoring-of-spontaneous-populations-of-genetically-modified-plant-species-in-the-environment.pdf.download.pdf/ig-gmo-2019-IG%20GMOs_Monitoring%20Genetically%20Modified%20Plants%20(1).pdf

Kortrijksesteenweg 127 B1
B 9830 Sint-Martens-Latem
Belgium

T +32 9 321 07 05
F +32 9 321 07 05
info@perseus.eu
www.perseus.eu

IBAN BE08 0013 9730 5713
BIC GEBABEBB

RPR Gent
VAT BE 0480 089 325