Taking stock of the environmental risk assessment of genetically modified plants and gene therapy

30 December 2015

Study performed for the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
Project 4410001148

Patrick L.J. RÜDELSHEIM & Greet SMETS
PERSEUS BVBA

Advisory Committee

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

Prof. dr. G.A.P. Hospers	Universitair Medisch Centrum Groningen
Dr. W.J. de Kogel	Wageningen University & Research Centre
Dr. I. de Kort Dr. J.E.E. Ng-A-Tham	Ministerie van Infrastructuur en Milieu (Ministry of Infrastructure and Environment)
Dr. D.C.M. Glandorf, Dr. H.C.M. van den Akker Dr. ir. M.M.C. Gielkens Dr. D.A. Bleijs Dr. ir. P.A.M. Hogervorst H. de Wijs	Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment)

Disclaimer

Summary

Based on the precautionary principle, legislation addressing environmental aspects of research on, development and marketing of genetically modified organisms (GMOs) was established in the early nineties. At that moment, limited knowledge on environmental effects of GMOs was available. Since then a vast amount of data has been generated. Both applicants and research institutes in Europe and elsewhere studied a diversity of aspects relating to environmental safety. In Europe and in the Netherlands in particular many field trials and clinical trials have been conducted. Much experience is gained on genetically modified (GM) crops that have been commercialised in several parts of the world. However, some topics may still lack sufficient high quality data. The National Institute for Public Health and the Environment (RIVM) commissioned PERSEUS bvba to inventory the areas of concern in environmental risk assessments (ERA) for development and market introduction, to evaluate the type of information necessary to perform ERAs both in the field of GM plants and gene therapy for humans, to identify areas in which our understanding has evolved to a level that provides confidence for conclusions in the ERA, to point out remaining areas of uncertainty and to recommend topics for further studies and initiatives.

A comparison of European legislation and legislation in other jurisdictions provides a basis for identifying the areas of concern in ERA. There are differences in the trigger to conduct an ERA mainly depending on the scope of organisms covered by the legislation and whether activities are considered contained use or not. Irrespective these differences, the methodology as well as the areas of concern are very comparable to what has been established in the EU:

- likelihood of the GMO to become more persistent and/or invasive than the recipient or parental organism;
- any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised;
- potential for gene transfer to other species and any selective advantage or disadvantage conferred to those species;
- potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable);
- potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens;
- possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s);
- possible immediate and/or delayed effects on animal health and consequences for the feed/food chain;
- possible immediate and/or delayed effects on biogeochemical processes;
- possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.

This convergence of methodology can be linked to the fact that most national and international regulations build on the criteria and principles set out in the Organisation for Economic Cooperation and Development (OECD) Blue Book on "Recombinant DNA Safety Considerations", published in 1986.

For GM plants all concerns are investigated. For gene therapy trials and products, the focus is predominantly on human health including that of the patient as well as staff and family members. Other environmental concerns are less addressed. Depending on the vector type some are deemed less relevant in gene therapy applications (*e.g.* effects on animals and consequences for the feed/food chain for human pathogen derived vectors, effects on biogeochemical processes for viral vectors as opposed to bacterial vectors, and effect of change in management techniques).

Some concerns voiced by stakeholders and the public at large may reflect important policy options, but they may not fit in the framework of an ERA. On the other hand, ERA could be further improved by agreement on protection goals and translating them in measurable assessment endpoints.

This report then presents an overview of available information that may serve to evaluate the different elements of the ERA for respectively GM plants and gene therapy:

- documents issued by governmental bodies (authorities, advisory committees);
- research commissioned by a governmental body;
- government funded research projects;
- consortium sponsored research;
- information from applicants; and
- scientific reports and publications, including monographs, reviews, meta-analyses, conferences and activities from societies.

This overview indicates that there is far more information relevant for ERA of GM plants than for the ERA on gene therapy. Possible factors influencing this bias include:

- prominent environmental exposure of GM plants;
- legal regime (focus on containment for gene therapy instead of environmental exposure);
- more advanced GM crop projects and products;
- global scale of GM crop products;
- responsive scientific community; and
- heightened critical public attention for GM plant applications.

With this "body of evidence", an attempt was made to identify elements for which ERA can be conducted with acceptable confidence and no additional information may be required as well as identifying areas in which research can be conducted to serve future ERA.

For GM higher plants:

- The comparative assessment acknowledges that the ERA of most activities with GMOs can build on what is already known for the non-modified host/parental organism. It recognises that the engineered modifications only change specific aspects of the organism. When the host organism has a history of safe use (or rather a use for which the impact is accepted), the ERA can focus on the potential impact resulting from the modification.
- It is questioned whether genes and gene constructs that were independently and repetitively assessed leading to the authorisation of different transformation events, should be subjected to a full assessment when deployed in new events. Similarly, experience obtained with stacked events should allow limiting the review to those cases where the stack is potentially leading to an interaction between the inserted traits.
- Major field crops are well documented and provide a model for other plant species. While the comparative method has been well established for field crops, it may proof to be difficult to apply for other plants (*e.g.* trees).
- Traits like specific herbicide tolerances and insect resistance have been elaborated and can serve as model for other traits. Looking ahead, modifications addressing essential aspects of the life cycle of the GM plant (*e.g.* modification of the reproductive biology) will potentially require adapted paradigms for evaluating invasiveness and/or persistence.
- Mechanism such as spread in the environment (seeds, pollen) and transfer to other organisms (pollen flow) have been documented in detail and further accumulation of information is unlikely to provide new insights for the risk assessment.
- The hypothetical concern for horizontal gene transfer can be neglected unless the trait would indicate a special safety issue.
- The interaction with other organisms, *i.e.* non-target organisms (NTO), has been studied in great detail for Bt proteins and more specifically Cry1Ab delta-endotoxin. Additional scientific research as well as experience from large scale release continues to broaden the range of NTOs exposed to these proteins and increases the level of confidence, but unless an unexpected counter-indication would be identified, there is no justification to expand the requirements for pre-authorisation NTO testing.
- Finally, given the diversity of existing agronomic practices, evaluating changes in management is largely influenced by the choice of the reference management regime.

For gene therapy:

- Only in specific gene therapy cases the parental organism has a history of safe use. Rather, in most applications, the non-modified parental organism is related to a pathogen, which can be strongly attenuated and/or to which the population may have built up immunity. The comparative approach must therefore identify the new intended modification; while at the same time confirm that pathogenic features have not been restored or that new ones have not been inadvertently introduced.
- Many types of vectors are used and for some frequently used vectors reviews are available. They provide a basis for the ERA and a model for new vectors. Concerns over negative effects originating from the vector seem to be focussed on aspects such as altered (cell) tropism, altered pathogenicity, survival capacity, and altered replication capacity. While these considerations are clearly related to possible concerns, the methodology to address them may not be standardised.
- The nature of the insert may change the assessment of the virus vector. Genes encoding cytokines, toxins or virulence factors will challenge the ERA. Given the multitude of inserts that have been used, a case-by-case approach is still needed.
- The release of a gene therapy product is not the purpose, but a consequence of its primary use, *i.e.* the treatment of the patient. Much attention has been given to "shedding" and may not be excluded. After the therapeutic injection local shedding can be expected. Also material may be accidentally spilled at the moment that samples are taken. When the vector is biodistributed through the blood vessels, shedding via urine, faeces and body fluids might be possible.
- Special attention is given to the transfer of the inserted genetic material to other organisms. This may occur due to integration of genetic material in the genome of the patient (or others exposed upon release in the environment) or via exchange of genetic material between the vector and organisms like viruses or bacteria. Recombination between the viral vector and wild-type viruses may lead to novel viral variants with different characteristics.
- The most important concern relates to the possibility for exchange of genetic information leading to gain of virulence functions and wild-type reversal. Given the diversity of vectors used so far, it seems too early to draw general conclusions.

The global experience illustrates that the stepwise, case-by-case approach has been successfully applied. Although it can be expected that for certain GMOs information will continue to be accumulated, this will not automatically lead to an improved ERA, rather further strengthen the supporting information. In this respect it can be argued that further elaboration of test systems and regulatory requirements may only present a perception of a better ERA. More fundamental progress of ERA for GMO as well as other stressors must be based on improved understanding of environmental interactions and on scientifically based problem formulation.

The experience gained so far confirms that GMOs are intrinsically neither more, nor less safe than other products. Taking stock of the ERA experience over 25 years can be a milestone in the discussion on whether the precautionary approach should specifically and solely be applied to organisms that are defined as "GMO".

Samenvatting

In de jaren ’90 van vorige eeuw werd wetgeving ontwikkeld rond milieuaspecten voor onderzoek, ontwikkeling en commercialisering van genetisch gemodificeerde organismen (ggo’s) gebaseerd op het voorzorgsprincipe. Op dat ogenblik was de kennis over milieueffecten van ggo’s nog beperkt. Sindsdien werd een omvangrijke hoeveelheid data gegenereerd. Zowel aanvragers als onderzoeksinstellingen in Europa en elders hebben een verscheidenheid aan milieuaspecten bestudeerd. In Europa en meer in het bijzonder in Nederland werden talrijke veldproeven en klinische proeven opgezet. Over de genetisch gemodificeerde (gg) gewassen die in vele delen van de wereld op de markt werden gebracht is veel ervaring is opgedaan. Toch zijn er nog onderwerpen die voldoende hoogkwalitatieve gegevens ontberen. Het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) gaf PERSEUS bvba de opdracht om de onderwerpen die van belang zijn in de milieurisicobeoordeling (MRB) voor ontwikkeling en commercialisering op te lijsten, om de aard van de informatie die nodig is voor de uitvoering van een MRB zowel voor gg-planten als gentherapie bij mensen te evalueren, om die onderwerpen te identificeren waarvoor het kennisniveau dusdanig is geëvolueerd dat er met vertrouwen conclusies kunnen getrokken worden in de MRB, om onderwerpen aan te duiden waarover nog onzekerheid bestaat en om aanbevelingen te doen voor verdere studies en initiatieven.

Een vergelijking van de Europese wetgeving en die in andere jurisdicties biedt een basis om de onderwerpen die van belang zijn in de MRB vast te stellen. De aanleiding om een MRB uit te voeren kan verschillen naargelang de aard van de organismen die de wetgeving behelst en of activiteiten als ingeperkt gebruik worden beschouwd of niet. Ondanks die verschillen zijn de methoden zowel als de aandachtspunten zeer vergelijkbaar met wat in de EU is vastgesteld:

- waarschijnlijkheid dat het ggo persistenter en/of invasiever wordt dan het recipiënte of ouderorganisme.
- selectieve voordelen of nadelen die op het ggo worden overgedragen en de waarschijnlijkheid dat dit daadwerkelijk tot uitdrukking komen.
- kans op genoverdracht op andere soorten en selectieve voordelen of nadelen die op deze soorten worden overgedragen.
- mogelijke onmiddellijke en/of vertraagde milieueffecten van de directe en indirecte interacties tussen het ggo en doelwitorganismen (indien van toepassing).
- mogelijke onmiddellijke en/of vertraagde milieueffecten van de directe en indirecte interacties tussen het ggo en niet-doelwitorganismen, inclusief de effecten op de populatie van concurrenten, prooien, gastheren, symbionten, predatoren, parasieten en ziekteverwekkers.
- mogelijke onmiddellijke en/of vertraagde effecten op de menselijke gezondheid van mogelijke directe en indirecte interacties tussen het ggo en personen die werken met, in contact komen met of in de nabijheid komen van de ggo-introductie(s).
- mogelijke onmiddellijke en/of vertraagde effecten op de gezondheid van dieren en effecten op de voeder/voedselketen.
- mogelijke onmiddellijke en/of vertraagde effecten op biogeochemische processen.
- mogelijke onmiddellijke en/of vertraagde, directe en indirecte milieueffecten van de specifieke technieken die voor het beheer van de ggo's worden gebruikt, indien deze verschillen van de voor niet-ggo's gebruikte technieken.

De overeenkomsten in de methodologie kunnen worden teruggevoerd op het feit dat nationale en internationale wetgeving gebaseerd is op de criteria en principes zoals uiteengezet in het Blue Book over "Recombinant DNA Safety Considerations" van de Organisatie voor Economische Samenwerking en Ontwikkeling (OESO), gepubliceerd in 1986.

Voor gg-planten werden alle onderwerpen van belang onderzocht. Voor gentherapieproeven en producten ligt de focus hoofdzakelijk op de menselijke gezondheid van zowel de patiënt als de zorgverleners en familieleden. Andere milieuaspecten zijn minder besproken. Afhankelijk van het type vector worden sommige minder relevant gevonden in gentherapie (vb. effecten op dieren en gevolgen voor de voeder/voedselketen wanneer een vector werd afgeleid van een humane pathogeen, effecten op biogeochemische processen v.w.b. virale vectoren (in tegenstelling tot bacteriële vectoren), en effecten van gewijzigde beheerstechnieken).

Sommige bezorgdheden vanwege belanghebbenden en het publiek in het algemeen kunnen belangrijke beleidsopties betekenen, maar passen mogelijk niet in het kader van een MRB. Aan de

andere kant kunnen MRBs verder worden verbeterd wanneer overeenstemming wordt bereikt over beschermingsdoelen vertaald in meetbare evaluatie-eindpunten.

Dit rapport geeft een overzicht van de beschikbare informatie die kan dienen om de diverse elementen in een MRB te evalueren voor respectievelijk gg-planten en gentherapie:

- documenten uitgegeven door de overheid (autoriteiten, adviesorganen);
- onderzoek in opdracht van de overheid;
- onderzoeksprojecten gesponsord door de overheid;
- onderzoek gesponsord door consortia;
- informatie van de aanvragers;
- wetenschappelijke rapporten en publicaties, waaronder monografieën, overzichtsartikels, meta-analyses, symposia en activiteiten van verenigingen.

Dit overzicht duidt aan dat er veruit meer relevante informatie beschikbaar is voor de MRB van gg-planten dan voor de MRB van gentherapie. Mogelijke factoren die deze scheeftrekking veroorzaken:

- prominente blootstelling van het milieu aan gg-planten;
- wettelijk kader (focus op inperking voor gentherapie i.p.v. milieublootstelling);
- verder gevorderde projecten en producten van gg-gewassen;
- wereldwijde reikwijdte van gg-gewasproducten;
- alert reagerende wetenschappelijke gemeenschap; en
- verhoogde kritisch publieke waakzaamheid voor toepassingen van gg-planten.

Met deze duidelijke aanwijzingen werd een poging ondernomen om elementen aan te duiden waarvoor een MRB met enige zekerheid kan concluderen dat geen verdere informatie is vereist, zowel als om onderwerpen te identificeren voor onderzoek die nuttig kunnen zijn voor toekomstige MRB.

Voor hogere gg-planten:

- Een vergelijkende beoordeling bevestigt dat de MRB voor de meeste activiteiten met ggo's kan bouwen op de kennis die er al is voor het niet-gemodificeerde gastheer/ouderorganisme. Deze werkwijze erkent dat de aangebrachte modificaties slechts specifieke aspecten van het organisme wijzigt. Als het gastheerorganisme een geschiedenis van veilig gebruik (of eerder een gebruik waarvan de impact acceptabel is) kent, kan de MRB focussen op de mogelijke impact van de modificatie.
- Er wordt betwijfeld of genen en genconstructen, waarvoor al herhaaldelijk en onafhankelijk MRB werden uitgevoerd die geleid hebben tot toelatingen van diverse transformatiegebeurtenissen, opnieuw een volledige beoordeling moeten ondergaan wanneer ze in nieuwe transformatiegebeurtenissen worden toegepast. Evenzo zou ervaring opgedaan met gestapelde gebeurtenissen moeten toelaten dat alleen die gevallen waar de stapeling van genen mogelijk kan leiden tot interactie van de inserties zouden worden beoordeeld.
- De grote veldgewassen zijn goed beschreven en zijn een model voor andere soorten. Hoewel de vergelijkende beoordeling goed is ingeburgerd voor veldgewassen, kan de toepassing ervan moeilijk blijken voor andere gewassen (vb. bomen).
- Eigenschappen zoals specifieke herbicidetolerantie en insectresistentie zijn goed uitgewerkt en kunnen dienen als voorbeeld voor andere eigenschappen. Vooruitkijkend kunnen modificaties die de essentiële aspecten van de plantenlevenscyclus bepalen (vb. modificatie van de voortplanting) mogelijk aangepaste paradigma's vereisen om invasiviteit en persistentie te beoordelen.
- Mechanismen zoals verspreiding in het milieu (zaden, pollen) en overdracht naar andere organismen (pollendrift) werden in detail beschreven en het is onwaarschijnlijk dat het verder verwerven van informatie nieuwe inzichten zal bijdragen aan de MRB.
- Het hypothetische bezorgdheid voor horizontale gentransfer is verwaarloosbaar tenzij een bepaalde eigenschap een speciaal veiligheidsrisico zou inhouden.
- Interactie met andere organismen, nl. de niet-doelwitorganismen, is uitgebreid bestudeerd voor de Bt-eiwitten, meer specifiek voor het Cry1Ab delta-endotoxine. Bijkomend wetenschappelijk onderzoek zowel als de ervaring opgedaan bij wereldwijde introducties doet voortdurend het aantal niet-doelwitorganismen dat wordt blootgesteld aan deze eiwitten toenemen en verhoogt het vertrouwen. Behalve wanneer onverwacht het tegendeel wordt bewezen, is het niet verantwoord om de eisen voor het testen van niet-doelwitorganismen voorafgaand aan een autorisatie te verstrengen.

- Ten slotte, gegeven de verscheidenheid van bestaande landbouwpraktijken is de beoordeling van gewijzigd beheer in grote mate afhankelijk van de gekozen referentiepraktijk.

Voor gentherapie:

- Slechts in enkele gevallen heeft het ouderorganisme een geschiedenis van veilig gebruik. In de meeste toepassingen is het niet-gemodificeerd ouderorganisme eerder gerelateerd aan een pathogeen, dat weliswaar sterk geattenueerd kan zijn en/of waartegen de populatie immuniteit heeft opgebouwd. De vergelijkende benadering moet daarom de nieuwe, bedoelde modificaties identificeren, en tegelijkertijd bevestigen dat de pathogene eigenschappen niet werden hersteld of dat geen nieuwe onbedoeld werden ingebracht.
- Er worden vele soorten vectoren gebruikt en voor sommige frequent gebruikte vectoren zijn overzichten beschikbaar. Ze bieden een basis voor de MRB en een voorbeeld voor nieuwe vectoren. Bezorgheden over negatieve effecten vanuit de vector zijn gefocust op aspecten zoals een gewijzigd (cel)tropisme, een gewijzigde pathogeniciteit, overlevingscapaciteit en een gewijzigde mogelijkheid tot replicatie.
- De aard van de insertie kan de beoordeling van de virusvector veranderen. Genen die coderen voor cytokines, toxines of virulentiefactoren zullen een uitdaging zijn voor de MRB. Gegeven de veelheid aan gebruikte inserties zal een geval-per-geval beoordeling nog steeds noodzakelijk zijn.
- Meestal is de introductie in het milieu van een gentherapieproduct niet het doel, maar het gevolg van het gebruik, nl. de behandeling van de patiënt. Er werd veel aandacht besteed aan “shedding”. Dit mag niet worden uitgesloten want na het toedienen van het therapeuticum kan er lokale lekkage worden verwacht. Materiaal kan ook per ongeluk worden gemorst op het ogenblik van monstername. Wanneer biodistributie de vector naar bloedbanen leidt, kan “shedding” optreden via de urine, feces en lichaamsvochten.
- Speciale aandacht gaat naar de transfer van ingebracht genetisch materiaal naar andere organismen. Dat kan te wijten zijn aan integratie van het genetisch materiaal in het genoom van de patiënt (of anderen die eraan werden blootgesteld) of gebeuren via uitwisseling van genetisch materiaal tussen de vector en organismen zoals virussen en bacteriën. Recombinatie tussen de virale vector en wildtype virussen kan leiden tot nieuwe varianten met andere eigenschappen.
- Het meest belangrijke punt van bezorgdheid houdt verband met de mogelijkheid dat uitwisseling van genetische informatie leidt tot het herwinnen van de virulentiefuncties en teugkeer naar het wildtype. Door de diversiteit van de gebruikte vectoren lijkt het te vroeg om al algemene conclusies te trekken.

De wereldwijde ervaring leert dat de stapsgewijze, geval-per-geval benadering met succes werd toegepast. Hoewel kan worden verwacht dat er voor zekere ggo’s informatie zal blijven aangeleverd worden, zal dit niet automatisch leiden tot een betere MRB, maar eerder de onderbouwing verstevigen. In dit opzicht kan er worden beargumenteerd dat verdere uitwerking van testmethoden en wettelijke vereisten alleen maar een perceptie van een betere MRB betekenen. Een meer fundamentele vooruitgang van de MRB voor ggo’s zowel als andere stressoren moeten worden gebaseerd op een beter begrip van interacties in het milieu en op wetenschappelijk gebaseerde probleemstelling.

De ervaring tot dusver bevestigd dat ggo’s intrinsiek niet meer, noch minder veilig zijn dan andere producten. De balans opmaken van meer dan 25 jaar MRB-ervaring kan een mijlpaal zijn in de discussie of de benadering volgens het voorzorgsprincipe specifiek en alleen moet worden voorbehouden aan organismen die gg worden genoemd.

Table of contents

Abbreviations

AAV	*Adeno-associated virus*
AMIGA	Assessing and Monitoring the Impacts of Genetically modified plants (GMPs) on Agro-ecosystems
APHIS	Animal and Plant Health Inspection Service (USA)
APVMA	Australian Pesticides and Veterinary Medicines Authority
BLA	Biologics License Application (USA)
Bt	*Bacillus thuringiensis*
CEPA	Canadian Environmental Protection Act
COGEM	Netherlands Commission on Genetic Modification
DEFRA	Department for Environment, Food & Rural Affairs (UK)
DIR	dealings involving intentional release (Australia)
EA	environmental assessment (USA)
EFSA	European Food Safety Authority
EIGMO	Ecological Impact of Genetically Modified Organisms
EIS	Environmental Impact Statement (USA)
EMA	European Medicines Agency
EPA	Environmental Protection Agency (USA)
ERA	environmental risk assessment
ERGO	Ecology Regarding Genetically Modified Organisms
F&DA	Food and Drugs Act (Canada)
FDA	Food and Drug Administration (USA)
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (USA)
FONSI	finding of no significant impact (USA)
FP	Framework Programme
gg	“genetisch gemodificeerd”
ggo	“genetisch gemodificeerd organisme”
GM	genetically modified
GMHP	genetically modified higher plant
GMO	genetically modified organism
GMP	genetically modified plant
GPG	general protection goal
GRACE	GMO Risk Assessment and Communication of Evidence
GURT	genetic use restriction technology
hGM-CSF	gene encoding human Granulocyte-Macrophage Colony-Stimulating Factor
HSE	Health and Safety Executive (UK)
HT	Herbicide tolerance/tolerant
ICGEB	International Centre for Genetic Engineering and Biotechnology
IOBC	International Organization for Biological Control of noxious animals and plants
ISBGMO	International Symposium on Biosafety of Genetically Modified Organisms
ISBR	International Society for Biosafety Research
JRC	Joint Research Centre
LMO	living modified organism
MEA	Millennium Ecosystem Assessment
MRB	“milieurisicobeoordeling” (environmental risk assessment)
MVA	*modified vaccinia virus Ankara*
NEPA	National Environmental Policy Act (USA)
NSNR	New Substances Notification Regulations (Canada)
NTA	non-target arthropod
NTO	non-target organism
OECD	Organisation for Economic Cooperation and Development
OGTR	Office of the Gene Technology Regulator (Australia)
PBO	Plant Biosafety Office (Canada)
PIP	Plant-Incorporated Protectants
PMEM	Post-Market Environmental Monitoring
PMM	Post-Market Monitoring
PNT	plant with novel traits

PPP	plant protection product
RIVM	“Rijksinstituut voor Volksgezondheid en Milieu” (National Institute for Public Health and the Environment)
SCAR	Standing Committee on Agricultural Research
SIN	self-inactivating
SNIF	Summary Notification Information Format
SPG	specific protection goal
WHV	*Woodchuck Hepatitis Virus*
WPRE	Woodchuck Hepatitis Virus posttranscriptional regulatory element
WPRS	West Palearctic Regional Section of the IOBC

1 Introduction

Research on, development and marketing of genetically modified organisms (GMOs) have to be performed in compliance with the applicable legal requirements. In the European Union (EU) specific legislation was established in 1990 with Directive 90/219/EEC[1] on contained use and Directive 90/220/EEC[2] on deliberate release of GMOs. These directives stipulate that in order to guarantee safety for humans, animals and the environment an environmental risk assessment (ERA) shall be performed before any activity can start. Subsequently the legal requirements were further elaborated and clarified leading to respectively Directive 2009/41/EC[3] and Directive 2001/18/EC[4].

In Directive 2001/18/EC an environmental risk assessment is defined as (Art. 2.1(8)):

> *'the evaluation of risks to human health and the environment, whether direct or indirect, immediate or delayed, which the deliberate release or the placing on the market of GMOs may pose and carried out in accordance with Annex II'.*

The purpose is further explained in Annex II:

> *'The objective of an ERA is, on a case by case basis, to identify and evaluate potential adverse effects of the GMO, either direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have. The ERA should be conducted with a view to identifying if there is a need for risk management and if so, the most appropriate methods to be used.'*

The environment that has to be taken into account is not defined in European legislation. However, the Netherlands Commission on Genetic Modification (COGEM) in a topical report defines environment in this context as:

> *'dat deel van de biotische en abiotische omgeving dat buiten het toepassingsgebied van de voorgenomen activiteit met een ggo ligt' (CGM/141222-02) (that part of the biotic and abiotic environment that is outside the scope of the intended GMO activity).*

Also,

> *'Door bovengenoemde afhankelijkheid van het toepassingsgebied van het ggo constateert de COGEM dat de reikwijdte van het milieu voor ieder type gebruik van ggo's verschillend is.' (Because of this dependence on the scope, the extent of the environment is different for each type of use of the GMO).*

Typically, an ERA is a 6-step process (Directive 2001/18/EC Annex II) as presented in Figure 1. In the first step potential harmful characteristics or hazards are identified. A 'hazard' is defined as the potential of an organism to cause harm to or adverse effects on human health and/or the environment (Commission Decision 2002/623/EC[5]).

Potential adverse effects of GMOs may include:

- disease to humans including allergenic or toxic effects,
- disease to animals and plants including toxic, and where appropriate, allergenic effects,

[1] Council Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms. OJ L117, 8.5.1990, p.1-14.

[2] Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms. OJ L117, 8.5.1990, p.14-27.

[3] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. OJ L125, 21.5.2009, p.75-97.

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L106, 17.4.2001, p.1-38.

[5] Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L200, 30.7.2002, p.22-33.

- effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations,
- altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors, compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes conferring resistance to antibiotics used in human or veterinary medicine,
- effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material.

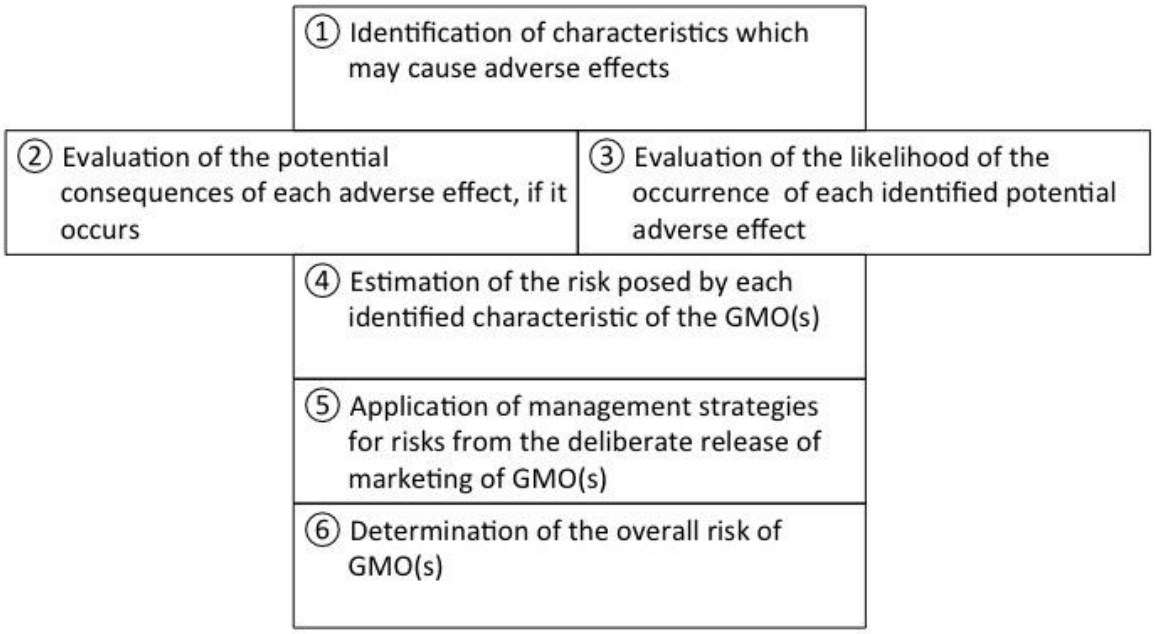

Figure 1 The six steps in the analysis of ERA (source Commission Decision 2002/623/EC)

The second step assesses the magnitude of the consequences of each potential adverse effect. Step 3 is often performed simultaneously and involves the evaluation of the likelihood of the occurrence of each identified potential adverse effect. The 'risk' is then the combination of the magnitude of the consequences of a hazard, if it occurs, and the likelihood that the consequences occur (step 4). When important risks are identified, measures to avoid them can be determined in the risk management plan, *i.e.* by changing the activities in such a way that the hazard or the likelihood is reduced, or to mitigate the potential impact (step 5). Step 6 determines the residual risk taking into account these risk management strategies.

The development of genetically modified (GM) plants is a gradual process (step-by-step) in which a project typically evolves from early research in contained environment, over greenhouse trials and limited field trials to larger, multi-location trials and marketing. Likewise gene therapy products are developed starting with an assessment in confined laboratories, evolving over animal trials and human clinical trials before they may reach the market. Each step is an opportunity to gather information on possible effects. Completing each step is a prerequisite for taking further steps: the next step is only allowed if sufficient information is available to conduct an ERA for the next step, where necessary complemented with management measures. At the same time the extent of the exposed environment and number of people involved is increasing at each step.

Based on the precautionary principle, the legislation was established in the early nineties with limited knowledge on environmental effects of GMOs. Since then a vast amount of data has been generated. Both applicants and research institutes in Europe and elsewhere studied a diversity of aspects relating to environmental safety. In Europe and in the Netherlands in particular many field trials and clinical trials have been conducted. Much experience is gained on GM crops that have been commercialised in several parts of the world. However, some topics may still lack sufficient high quality data.

The National Institute for Public Health and the Environment (RIVM) commissioned PERSEUS bvba to inventory the areas of concern in ERAs for market introduction, to evaluate the type of information necessary to perform ERAs both in the field of GM plants and gene therapy for humans[6], to identify areas in which our understanding has evolved to a level that provides confidence for conclusions in the ERA, to point out remaining areas of uncertainty and to recommend topics for further studies and initiatives.

Chapter 2 explores European legislation and legislation in other jurisdictions and compares the areas of concern that are used in ERA globally. In Chapter 3 an overview is presented of available information that may serve to evaluate the different elements of the ERA. Following a discussion on the type of available information in Chapter 4, elements are identified where research results are available to confidently answer ERA questions. The fifth Chapter points to uncertainties and the fields that can benefit from further research. In the last chapter conclusions are formulated.

[6] Part IV of Annex I to Directive 2001/83/EC defines "gene therapy medicinal product" as a biological medicinal product which has the following characteristics:

(a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;

(b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products shall not include vaccines against infectious diseases.

Applications in veterinary medicine and animal health have been excluded from the scope of this report.

2 ERA hypothesis and areas of concern

2.1 European Union

The broad goal of protecting human health and the environment is a basic principle embedded in the Treaty of the European Union. Art. 3.3 says:

> *'The Union […] shall work for the sustainable development of Europe based on balanced economic growth and price stability, a highly competitive social market economy, aiming at full employment and social progress, and a high level of protection and improvement of the quality of the environment … '*

This policy statement is further elaborated in EU legislation like the Habitats Directive 92/43/EEC[7]; the Water Framework Directive 2000/60/EC[8], Directive 2008/50/EC on ambient air quality[9], the Birds Directive 2009/147/EC[10], Regulation (EC) No 1907/2006[11] (REACH), Regulation (EC) No 1107/2009 on plant protection products[12] etc. (EFSA, 2010a).

Protecting these natural resources means preventing and mitigating damage to them. The European Directive 2004/35/EC on Environmental Liability[13] defines damage in Art. 2.2:

> *'damage' means a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly'*

Still these policies present general protection goals that need further specification in order to become operational. Ideally legislation specifically mentions what needs to be protected by detailing specific protection goals (SPG). In the context of this report an example of a SPG would be: "representative bacterial populations in the rhizosphere of the GM plant during the cropping season." Problem formulation allows for formulating the risk hypotheses that need to be tested (Raybould, 2006, García-Alonso & Raybould, 2014). An example of the latter would be: "the genetic modification has not resulted in potentially harmful changes in the relative abundance of bacterial populations in the rhizosphere of particular GM plants." Problem formulation including the characterisation of the GMO and the formulation of risk hypotheses is the first step in the ERA.

SPGs may concern *e.g.* certain animal species that are valued by the public such as an endangered bird species. They might as well represent ecosystem services. These ecosystem services are the benefits people obtain from ecosystems. These include provisioning services such as food and water; regulating services such as regulation of floods, drought, land degradation, and disease; supporting services such as soil formation and nutrient cycling; and cultural services such as recreational, spiritual, religious and other nonmaterial benefits (MEA, 2005). Ecosystem services related to GM plants can be the regulating service delivered by

---

7 Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. OJ L206, 22.7.1992, p.7-50.

8 Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. OJ L327, 22.12.2000, p.1-72.

9 Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. OJ L152, 11.6.2008, p.1-44.

10 Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds. OJ L 20, 26.1.2010, p.7-25.

11 Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L396, 30.12.2006, p.1- 849.

12 Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L309, 24.11.2009, p.1-50.

13 Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage. OJ L143, 30.4.2004, p.56-75.

pollinators or by parasites and pathogens of crop pest organisms. In that case the ERA considers the impact on the ecosystem service rather than de effect on a single species.

In European legislation no SPGs for environmental risk assessment of GMO are established. This leaves the decision on what needs to be protected to the risk assessors. Although 'hazard' and 'risk' are defined (Commission Decision 2002/623/EC), the 'limits of concern' defined as the minimum ecological effects that are deemed biologically relevant and that are deemed of sufficient magnitude to cause harm (EFSA, 2010a) are not set.

To allow a pragmatic approach to the ERA regarding the use of GMOs, Annex II of Directive 2001/18 formulates 'areas of concern' both for genetically modified higher plants (GMHP) and non-GMHP GMOs. An applicant must address these areas in the ERA when applying for an activity with a GMO. The implementing Commission Decision 2002/623/EC and guidance documents by the European Food Safety Authority (EFSA) give further direction as to what applicants of GMO activities need to consider (EFSA, 2010a; EFSA, 2010c).

In the Member States the details on how to perform an ERA including the identification of areas of concern are either reiterated in national legislation or are provided as a reference to Directive 2001/18/EC. Examples of such national implementation, including the implementation in the Netherlands, are included in Annex 1. While there are no differences between countries in applying legal requirements for field trials and the commercialisation of GMHPs, the situation is different for gene therapy applications. In permitting clinical trials for humans some Member States follow the contained use legislation, while others handle the permit via the deliberate release legislation. The related ERA stresses therefore different safety and risk elements in association with different risk management requirements. On the contrary, commercial gene therapy applications are treated in the same way in each Member State.

Areas of concern for GMHP

For GMHP the areas of concern are summarised in part D2 of Annex II of Directive 2001/18. Each of the 9 areas is listed with a short explanation.

1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.
   A GMHP with increased fitness may lead to an increased volunteer management/weed problem due to more volunteer plants in the subsequent crop. Furthermore, when crossed with wild relatives, the progeny, if more persistent, may reduce the diversity/abundance of valued flora and fauna in semi-natural or natural habitats.

   An example of what may induce persistence and invasiveness is a higher seed/seedling vigour which may lead to an increased competing ability. Such a plant may replace other plants resulting in a decline in the local plant species community.

2. Any selective advantage or disadvantage conferred to the GMHP.
   If the GMHP has a selective advantage it may influence the relative success for establishing in specific environments compared with the conventional plant and with other plants. This may lead to a shift in biodiversity. Conversely a negative selective advantage can affect selectively a particular plant species or genotype.

   As an example a tolerance to a specific herbicide gives the GMHP a selective advantage in an agronomic setting where the corresponding herbicide is applied. If the same herbicide is used to control weeds in other crops and/or other managed areas, the surviving GMHP may present a new challenge. Similarly traits providing GMHP mechanisms to overcome limiting factors (*e.g.* disease resistance, drought resistance) may potentially give a selective advantage expanding the possible habitat.

3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.

Depending on the species, introduced traits can be transferred to sexually compatible species. The traits can then show a similar or a new selective advantage/disadvantage. This will only be realised when hybridisation is possible, and hybrids are viable, are able to set seed, survive the winter etc.

When the herbicide tolerance gene is transferred to wild relatives, the progeny may inherit the trait. In natural environments the trait gives no selective advantage as the herbicide is not applied. But if the wild relative presents a weed problem in crops, the introgressed trait may worsen the situation.
Similarly, traits like disease resistance, drought resistance etc. are characteristics that when outcrossed to wild relatives may potentially give the progeny a selective advantage. However, as wild relatives are better adapted to a diversity of environmental conditions than domesticated crop plants, the additional trait -though important for crop performance- may have little effect on the fitness of the wild relative.

While the Annex only lists transfer to sexually compatible plant species, also gene transfer from plant to micro-organisms has been raised as an area of concern. Plants decay and DNA may end up in the soil in contact with soil micro-organisms. Likewise, in animals feeding on the GM plant micro-organisms inside the gut may take up cell free DNA. Bacteria are capable of exchanging genetic material directly between each other via conjugation, transduction or transformation, and the acquired DNA sequence may spread in this way. Therefore, the likelihood of plant DNA uptake by micro-organisms and integration into their genomes needs to be analysed. Although a rare event – integration into the genome happens mainly by homologous recombination requiring homologous sequences in plant and micro-organisms - the potentially acquired trait and the prevalence of similar traits in microbial communities will determine further consequences. *E.g*. the trait of resistance to the antibiotic kanamycin is ubiquitous in soil organisms. Acquired from the DNA of a GMHP this characteristic will not add much to the microbial environment. Alternatively, if resistance to an antibiotic used in human or animal medicine reaches pathogenic organisms, this may hamper medical treatment.

4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).
   Some GM plants are designed to resist certain target organisms, such as pathogens (bacterial, viral or fungal diseases) and pests (nematodes, mite, snails, slugs, and insects). Certain scenarios of wide scale exposure of populations of the target organism to these resistant plants may lead to the selection and increase in numbers of resistant individuals. The target population becoming resistant renders the protection ineffective.

   Although this is predominantly an agronomic concern, it is also seen as an environmental aspect as it would impact a supposedly environmental friendly solution and may lead to the use of more/other plant protection products. Strategies need to be designed to delay or prevent the occurrence of pest resistance as part of a response to both the environmental as well as the agronomic concern.

5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.
   All other organisms not targeted by the GMHP are considered non-target organisms (NTO). The GM crop may have a potential environmental impact, direct or indirect, on biodiversity in general. Several functions offered by a diversity of organisms may be affected such as pollinators, herbivores, natural enemies, symbionts, parasites and pathogens, plant material degrading organisms, organisms involved in nutrient cycling etc. Pollinators, natural enemies and plant symbionts like bacteria in the rhizosphere, may be affected as a side-effect of the expression of an active substance with a broad spectrum impact. The plant may have become more resistant to decomposers hampering the recycling of nutrients that is important for soil fertility.

Effects on target organisms will also impact the food web to which the target organisms contribute: predators, parasitoids and pathogens of the target organisms may therefore be affected as well. However, this will have to be compared with conventional cultivation where target organisms are controlled with plant protection products and by other techniques possibly also having an effect on NTO.

6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).
   Effects on human health might arise when people get exposed *e.g.* in direct contact with the GMHP (farmers) or when processing the crop products (food industry). Pollen and dust may have new adverse effects on humans compared to the non-GM counterpart *e.g.* when the newly expressed proteins would cause allergic reactions.

7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.
   While the actual food and feed use is regulated separately, the ERA takes into consideration animals directly feeding on the crops in the field and any products derived thereof. Although the consumption profile may be different, the same concerns are relevant relating to the intrinsic safety of the newly expressed proteins, presence of any anti-nutrient and any alteration in nutrient composition.

8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
   A biogeochemical cycle is a pathway by which a chemical substance moves through both biotic (biosphere) and abiotic (lithosphere, atmosphere, and hydrosphere) compartments of earth. While every living being contributes to these cycles, certain organisms present particular functions such as nutrient cycling, immobilisation and mobilisation of nutrients, decomposition of soil organic matter and emission of greenhouse gases. Some traits may change the role of the GMHP (*e.g.* if plants can be modified to fix nitrogen) or may influence other organisms (*e.g.* soil organisms contributing to a specific cycle).

9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.
   The introduction of a certain GM crop may change specific cultivation, management and harvesting techniques. This in turn may have an effect on the environment.

   Herbicide tolerance may lead to a no tillage practice which in its turn will affect soil organisms. Insect resistant plants can be applied in integrated pest management programmes. Still, other pests may become more important and this may require further adaptation of the programme.

Box 1 An example of the areas of concern summarised in the marketing authorisation of a genetically modified maize.

GMO: DAS-Ø15Ø7-1 a maize event resistant to certain lepidopteran species (*Ostrinia nubilalis* or *Sesamia nonagrioides*) and tolerant to herbicides based on glufosinate-ammonium
Reference: C/ES/01/01 (EFSA, 2005; Spanish Ministry of Environment, 2003).

Areas of concern:

- Persistence/ Invasiveness
  Maize is highly domesticated, not able to survive without the help of man. Maize lacks seed dormancy, has a low survivability of the seed in the soil, and is sensitive to frost. The genetic modification did not alter that and therefore the event is not expected to be persistent and invasive.

- Selective advantage/ disadvantage
  The GM maize has no selective advantage from the herbicide tolerance trait except when glufosinate-ammonium is applied. Lepidopteran resistance is not thought of as giving a selective advantage, because the main limiting factors to survive are the absence of dormancy, susceptibility to fungi and susceptibility to cold temperatures.

- Selective advantage/ disadvantage following transfer
  In Europe there are no sexually compatible plants for maize and therefore no gene transfer to other plant species is likely to occur. Crossing is only possible with other maize plants. But volunteers are very unlikely to establish, since the modification did not alter the fitness of maize as shown in several field trials.
  Although extremely unlikely, gene transfer to bacteria would occur primarily through homologous recombination. The genes, *cry1F* and *pat*, are derived from prokaryotic species and are widespread. Recombination would be possible but the genes are driven by eukaryotic promoters and would not be expressed in prokaryotic organisms. Even so, the traits would not present a selective advantage and are therefore not likely to become established.

- Effect on target organisms
  Resistance development against the Cry1F protein is possible due to the high selection pressure. Hence, this is monitored and taken up in the post-market environmental monitoring plan. Also, the planting of refuges will be mandatory.

- Effect on non-target organisms
  The population size of predators is expected to decrease due to the lack of prey species. However, this effect will be similar to the effect of the use of insecticides in conventional maize cultivation. The toxin itself is no risk to the predator. Due to the high specificity of the toxin other organisms are not likely to be affected as was shown in studies in non-target arthropods (NTA) (*Chrysoperla carnea*, *Hippodamia convergens*, *Danaus plexippus*, *Nasonia vitripennis*, etc.), bees (*Apis mellifera*), terrestrial organisms (*Eisenia foetida*, *Folsomia candida*), wildlife birds (*Colinus virginianus*) and aquatic organisms (*Daphnia magna*). Because *Bt* toxins are degraded rapidly in the gut, no accumulation higher in the food chain is expected.

- Effect on human health
  Information presented for approval of food/feed use, including animal feeding studies, was used to advise on effects due to contact and/or incidental consumption. Studies in mice (acute oral toxicity) or rats (90-day oral toxicity) with the Cry1F protein have demonstrated its safety to human and animal health. The same is true for PAT. The proteins do not pose any significant potential allergenic risk as shown in a comparative assessment with known allergens and due to the rapid degradation in simulated gastric fluid, relatively low expression level, lack of post-translational glycosylation and thermal susceptibility.

- Effect on animal health
  Concerning animal health the outcome is the same as above. Moreover, compositional analysis of protein, fibre, carbohydrates, fat, ash, minerals, vitamins, secondary metabolites, and anti-nutrients confirm the substantial equivalence with other non-GM maize.

- Effect on biogeochemical processes
  Plant litter, root exudates and pollen end up in the soil and the toxin is released. However, it is rapidly decomposed. Growth chamber studies found very few differences on soil microbial communities. The soil type revealed a greater variation in species abundance. A suggestion was made that a higher lignin content would delay biodegradation and mineralisation of plant litter, resulting in slower *Bt* toxin degradation. However, the compositional analysis of DAS-Ø15Ø7-1 did not show an altered lignification. Experiments with other *Bt* toxin producing

maize did not give conclusive evidence that they are causing significant direct effects on the soil environment.

- Agronomic techniques
  Glufosinate-ammonium is a contact, non-persistent and non-systemic broad-spectrum herbicide. Studies reveal that applying this herbicide compared to conventional herbicide regimes may have a better or equal biodiversity impact.

Areas of concern for gene therapy

When performing an ERA for GMOs destined for medicinal use, the 9 areas of concern listed in section D.1 "GMOs other than higher plants" of Annex II of Directive 2001/18 must be considered.

1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).
   Changes in host range, tissue tropism or a changed disease profile in the natural host may lead to a changed persistence of the gene therapy GMO compared to the natural vector organism. *E.g.* in order to treat a disease like cancer, recombinant viruses may be engineered to target tumour cells which the wild-type viruses would not normally infect. The specificity needs to be carefully checked in order not to affect other than the intended cells.

   Gene therapy GMOs are usually very host dependent for survival and therefore unlikely to invade other habitats. Nevertheless, there is a concern that following shedding the vector may infect other organisms.

2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release(s).
   A selective advantage or disadvantage may be conferred by the characteristics of the insert. Furthermore, all features of the GMO that increase the persistence and invasiveness may lead to a selective advantage.

3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.
   Due to homologous sequences the GMO may transfer DNA to other organisms. Recombination with wild-type versions of the GMO may result in more virulent strains. For example, replication-defective adenoviruses or conditionally replicative herpes viruses may undergo recombination with their wild-type counterparts that could reverse attenuating genetic lesions. This may result in more severe pathogenicity of the organism. Nonetheless, recombination is only possible when both organisms are present in the same compartment.

4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).
   In the context of gene therapy the target organisms are in most cases the patients. Their safety is covered by other legislation.

5. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.
   In gene therapy applications medical staff, family members and the broader public are considered to be the NTOs. People may come in contact when the GMO is spread into the environment, *e.g.* via wound leakage or waste. Their health may be affected by the GMO itself (*e.g.* if derived from a pathogen) or more often from the insert that may be toxic, or that induces allergenic effects (immediate effect) or is oncogenic (delayed effect).

   Depending on the host range of the gene therapy product, other NTOs may be relevant including animals (*e.g.* pets in the vicinity of the treated person). Irrespective, the scope of

NTOs seems to be *de facto* more limited than with GMHP. Also part is covered by other legislation (pharmaceutical products, workers protection).

6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).
   This item is already addressed with item 5, effects on NTOs.

7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.
   Reference is made to item 5. Animal health refers to the health of *e.g.* the patient's family pets or farm animals. The same effects as to humans may occur, when they belong to the GMO's host range. In human gene therapy applications the consequences for the food/feed chain are not relevant.

8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
   The viral vectors that are used in gene therapy have usually no particular role in biogeochemical processes. This may be different for bacterial vectors. The processes may be influenced indirectly *e.g.* when GMOs enter the sewage system or when waste is not processed correctly. Following infection or homologous recombination other organisms with an active role in nutrient cycles or decomposition of organic material may theoretically be affected.

9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.
   Concerning management gene therapy with a certain GMO may compromise prophylactic or therapeutic medical treatments. A decline in effect or decrease in applicability of medicines may be the result. This would be an important consideration when antibiotic resistance markers are used as may be the case with bacterial vectors or plasmids.

Box 2 An example of the areas of concern summarised in the marketing authorisation of a genetically modified gene therapy product.

GMO: Glybera (alipogene tiparvovec of uniQure biopharma B.V.) The GMO consists of an AAV-1 capsid with AAV-2 backbone expressing human lipoprotein lipase. Glybera is indicated for adult patients diagnosed with familial lipoprotein lipase deficiency.
Reference: EU/1/12/791(EMA, 2012a)

Areas of concern:

- Persistence/ Invasiveness
  Wild-type AAV does not cause human disease and can only replicate in the presence of a helper virus. The vector used in Glybera is replication defective and therefore will not compete with wild-type AAV and lacks the *rep* gene required for site-specific integration. Studies indicate that while 97% of the vector is maintained episomally a small proportion of the vector may integrate through non-homologous recombination into the chromosome, close to random.

- Selective advantage/ disadvantage
  The expressed gene, lipoprotein lipase, is naturally present in humans. There is no tumorigenic effect, based on information on the frequency and sites of vector insertion.

- Selective advantage/ disadvantage following transfer
  Shedding studies show that the vector may be shed from patients through urine (3-

4 weeks), faeces (to 8 weeks), saliva and seminal fluid (4-6 weeks). The dilution of any shed vector in waste water is likely to render any interaction with humans or animals unlikely and thus unlikely to result in transduction of humans or animals.
Homologous recombination with wild-type AAV-2 is only possible with the inverted terminal repeats (ITRs) and results in the loss of the expression cassette.
Baculovirus DNA fragments contained within the vector theoretically could recombine with baculovirus. The frequency is considered to be negligible. Also recombination with other sequences in the environment is not expected due to low numbers of Glybera released.
Non-homologous recombination into microbe genomes is possible, but very infrequent. The vector does not encode a microbial promoter that would result in expression or that would alter persistence or survival.
The possibility of shed DNA being incorporated by an animal or plant species is also considered to be remote and even if lipoprotein lipase were expressed it could not be further transmitted.

- Effect on target organisms
  Biodistribution studies found vector in muscle, local lymph nodes, liver and blood at high levels, and in brain, lung heart, gonads and reproductive organs and non-injected muscle groups at low levels. Data suggest that germ line transmission is unlikely, as the vector is not present inside the germ line cells.
  Baculovirus sequences present in Glybera are not transcribed and translated on transfection of muscle, liver or lymph nodes and thus present a negligible risk to those accidentally exposed to Glybera or to the environment.
  The Woodchuck Hepatitis Virus (WHV) posttranscriptional regulatory element (WPRE) expressing X protein may be associated with oncogenesis. However, in Glybera it is only partly present and therefore is not expressed, nor would it be expressed as a fusion protein with lipoprotein lipase due to a stop codon.

- Effect on non-target organisms
  Risk to humans other than the patient may follow from accidental injection of the product. This effect would be low as no full dose would be injected (0.01% of the number of particles injected into a patient) and injury would be percutaneous instead of the muscle with a low transduction frequency. Non-clinical and clinical data show that the risk of over-expression of lipoprotein lipase is low.
  WHV is not endemic to the European marmot species, but sequence similarity of Hepatitis B virus and WHV WPRE elements mean that the WHV WPRE sequence cannot be considered to be truly novel to Europe.
  Glybera is manufactured using a system of 3 recombinant baculoviruses in an insect cell line. The production process is reported to be capable of removing 10 logs of baculovirus. Animal studies used much higher doses and no toxicity was found

- Effect on human health
  (see effect on target organisms and effect on NTOs)

- Effect on animal health
  (see effect on NTOs)

- Effect on biogeochemical processes
  No specific concern was identified.

- Management techniques
  Glybera does not contain sequences that would interfere with prophylaxis or treatment of pathogens in humans, animals or plants.

2.2 Other approaches

2.2.1 USA[14]

GM plants

The Coordinated Framework for Regulation of Biotechnology describes the Federal system for evaluating products developed using modern biotechnology. It involves 3 federal agencies: the Animal and Plant Health Inspection Service (APHIS), Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). Existing law is used.

For APHIS the basis for regulation of agricultural biotechnology is the Plant Protection Act. It regulates certain genetic engineered organisms that may pose a risk to plant or animal health. The Code of Federal Regulation Volume 7, Section 340 (federal Plant Protection Act of 2000) says:

'Part 340 regulates, among other things, the introduction of organisms and products altered or produced through genetic engineering which are plant pests or which there is reason to believe are plant pests.'

The regulatory trigger is therefore the potentially harmful change to the characteristics of the organism that may lead to a plant pest. An organism is covered by the regulations if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in 7 CFR 340.2 (groups of organisms which are or contain plant pests), or is an unclassified organism, or any product or organism altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest, a "Plant Pest Risk Assessment" is conducted.

Other definitions include:

'Environment.
All the land, air, and water; and all living organisms in association with land, air and water.'

'Organism.
Any active, infective, or dormant stage or life form of an entity characterized as living, including vertebrate and invertebrate animals, plants, bacteria, fungi, mycoplasmas, mycoplasma-like organisms, as well as entities such as viroids, viruses, or any entity characterized as living, related to the foregoing.'

'Plant pest.
Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants.'

While deregulation decisions are based on the plant pest risk assessment, APHIS-USDA must also comply with the National Environmental Policy Act (NEPA). Depending on the situation, different types of evaluation are possible:

- Environmental Assessment (EA) are concise public documents that a Federal agency prepares under NEPA to provide sufficient evidence and analysis to determine whether a proposed agency action would require preparation of an Environmental Impact Statement (EIS) or a Finding Of No Significant Impact (FONSI).

[14] This section describes the approach established in the USA since 1986 and in use at the time of compiling the report. July 2015 an effort "to update the Coordinated Framework, develop a long-term strategy to ensure that the system is prepared for the future products of biotechnology, and commission an expert analysis of the future landscape of biotechnology products to support this effort." was announced. It is unclear if the announced update will have any impact on the information in this report.

- FONSIs are public documents issued by a Federal agency briefly presenting the reasons why an action for which the agency has prepared an environmental assessment will not have a significant effect on the human environment and, therefore, will not require preparation of an environmental impact statement.
- EISs are detailed written statements that are required by section 102(2)(C) of NEPA for a proposed major Federal action significantly affecting the quality of the human environment.

Field trials are permitted either through the notification system or via a permit.

- When the GM plant meets specified eligibility criteria and the introduction meets certain pre-defined performance standards the notification procedure may be followed. This is a streamlined administrative procedure where no explicit case-specific ERA has to be performed.
- Permits may require an EA or not.

Petitions for deregulation always require a case-by-case assessment. Depending on the circumstances, an EA and an EIS is prepared.

Environmental consequences as listed by NEPA are (especially sections 102(2)(C)(i), (ii), (iv), and (v) of the Act):

(i) the environmental impact of the proposed action,
(ii) any adverse environmental effects which cannot be avoided should the proposal be implemented,
(iii) alternatives to the proposed action,
(iv) the relationship between local short-term uses of man's environment and the maintenance and enhancement of long-term productivity, and
(v) any irreversible and irretrievable commitments of resources which would be involved in the proposed action should it be implemented.

The discussion will include the environmental impacts of the alternatives including the proposed action.

When GM plants contain genes for pest resistance, like insect resistance genes, these are regarded as Plant-Incorporated Protectants (PIPs) that fall under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)(40 CFR 174). The US Environmental Protection Agency (EPA) assesses the risks for human health and the environment.

When GM plants have PIPs the US EPA assesses:

- the risks to human health (toxicity, allergenicity, and skin and eye irritation, as well as long-term effects including cancer, birth defects, and reproductive and neurological system disorders),
- the risks to NTOs (birds, mammals, fish, invertebrates, and marine organism),
- the risks to the environment,
- the potential for gene flow, and
- the need for insect resistance management plans.

The Coordinated Framework policy is product-based. While it is true that process is a component of the regulatory trigger at APHIS (‘*organisms and products altered or produced through genetic engineering’*), APHIS’ assessments of the GMOs focusses on the biological properties of those organisms, not the method by which they were produced. EPA concentrates on pesticidal traits.
APHIS’ risk assessments are usually qualitative in nature, based on literature reviews and results from field testing. The risk assessments by EPA often consist of quantitative data. Stacked events are not considered by APHIS providing that each of the components has been deregulated. However, they are by EPA when they concern GMOs with 2 or more combined PIPs.

Box 3a An example of the areas of concern in a USDA Plant Pest Risk Assessment for the deregulation of a genetically modified crop.

GMO: glyphosate tolerant sugarbeet H7-1
Reference: USDA-APHIS, 2012a

The Plant Pest Risk Assessment discusses the:

- Potential for H7-1 to have altered disease and pest susceptibilities;
- Potential for effect on NTOs, including those beneficial to agriculture;
- Potential for enhanced weediness or invasiveness;
- Potential of H7-1 to impact the weediness of other plants with which it can interbreed;
- Potential changes to agricultural or cultivation practices;
- Potential impacts from transfer of genetic information to organisms with which H7-1 cannot interbreed.

Box 3b An example of the areas of concern in a USDA Final Environmental Impact Statement for the deregulation of a genetically modified crop.

GMO: glyphosate tolerant sugarbeet H7-1
Reference: USDA-APHIS, 2012b

After a thorough description of the affected environment (production and management of beet crops, biological resources, socio-economics, the physical environment and human health and safety) the environmental consequences are scrutinised:

- Production and management of beet crops
- Biological resources (animals, micro-organisms and plants)
- Socio-economic impacts (sugar, sugarbeet and seed markets)
- Effects on the physical environment (land, soil, air, climate change (*e.g*. production of greenhouse gas), surface and groundwater quality)
- Human health and safety (public health and safety, worker health and safety)
- Other impacts (unavoidable impacts, short-term vs. long-term productivity of the environment, irreversible resource commitments)

Also cumulative effects are studied at the local, regional and national level.

Box 3c An example of the areas of concern in a USDA Environmental Assessment for the deregulation of a genetically modified crop.

GMO: potatoes with low acrylamide potential and reduced black spot bruise (Simplot Innate™ potato)
Reference: USDA-APHIS, 2014

The EA takes into consideration:

- Environmental Considerations:
  - Water Resources
  - Soil
  - Air Quality
  - Climate Change
  - Animals
  - Plants
  - Gene Flow
  - Microorganisms
  - Biological Diversity
- Human Health Considerations:
  - Public Health
  - Worker Safety
- Livestock Health Considerations:
  - Livestock Health/Animal Feed

Box 3d An example of the areas of concern in an EPA PIP assessment for the marketing authorisation of a genetically modified crop.

GMO: Cry1Ac protein in MON 877Ø1-2 soybean
Reference: EPA, 2010

Mammalian toxicity and allergenicity of the protein, and endocrine disruptors are topics for human health. Concerning the environment a tiered approach is used to study toxicity to NTOs (birds, mammals, non-target insects, like honey bees, plants, and aquatic species, and especially endangered species), and exposure to the protein. Also the fate of the protein in the soil (degradation), effects on soil micro-organisms, horizontal gene transfer to these organisms, gene flow and weediness potential are mentioned.

Gene therapy

The Food and Drug Administration (FDA) handles gene therapy applications as any other medicinal product under the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 CFR). For clinical trials the gene therapy product is applied for as an investigational new drug (IND) (21 CFR 312). Once trials are completed successfully the sponsor submits a New Drug Application (NDA) or in the case of a gene therapy product this would be a Biologics License Application (BLA) (21 CFR 601.2).

FDA's primary task is to ensure the quality and safety of gene therapy products and that these products are properly studied in human subjects. Under NEPA FDA is to assess the environmental impacts of its actions and to ensure that the interested and affected public is informed of environmental analyses.

An IND is excluded from this assessment, and, therefore, normally does not require the preparation of an Environmental Assessment (EA) or an Environmental Impact Statement (21 CFR Part 25 Subpart C-Categorical Exclusions, Sec. 25.31 Human drugs and biologics). According to FDA a clinical trial with an IND does not individually or cumulatively have a significant effect on the human environment. Generally relatively small quantities of a drug or biologic product are involved and only a limited number of patients are treated. Likewise the IND safety reports (21 CFR 312.32) only relate to the patient or subject in the clinical trial (Investigational New Drug Safety Reporting Requirements for Human Drug and Biological Products and Safety Reporting Requirements for Bioavailability and Bioequivalence Studies in Humans (Federal Register /Vol. 75, No. 188, September 29, 2010 /Rules and Regulations, 59935)).

When extraordinary circumstances exist at least an EA is necessary (21 CFR Part 25.21). Such exceptions include applications which require the use of cytotoxic substances or when large volumes of waste are produced. Also, an EA is usually required for use of virulent organisms, organisms that are ecologically more fit than their wild-type counterparts, or organisms for which eradication is problematic or difficult to document. Where significant adverse effects cannot be avoided, FDA uses the submitted information as the basis for preparing an Environmental Impact Statement.

Concerning the risks for healthcare workers the National Institute for Occupational Safety and Health, as part of the Centers for Disease Control and Prevention (CDC) in the Department of Health and Human Services is responsible.

For market applications (NDA) an EA is usually required. In case of a gene therapy product a BLA also requires an EA unless exempt. The most important item is toxicity to organisms in the environment (fate and effect testing) (FDA, 1998).

The Guidance for Industry (FDA, 2015a) explains when an EA is necessary and, if so, how to perform an EA and which items need to be addressed. Topics include:

- Identifying potential environmental effects
    - Phenotypic attributes of the parental strain and/or vector:
        - Is the strain or vector virulent, pathogenic, or known to be associated with animal, plant or microbial toxicities?
        - Is there an understanding of the environmental distribution, host range, and tropism?
        - Are there substrates that may limit growth or reproduction?

        - Is the strain or vector susceptible to control by antibiotics, antivirals, or biocides?
        - What is known regarding the genetic stability and prevalence of gene exchange in natural populations of the strain or vector?
        - What is known about the stability of the strain or vector in the environment and is the strain capable of survival under adverse conditions (spores, dormancy, etc.)?
    - Environment into which the GTVV[15] may be introduced:
        - Does the product have traits that may give it a selective advantage over natural organisms?
        - Would susceptible species be exposed?
        - Does the environment provide limited or reduced capacity for growth or reproduction?
        - Are species or strains closely related to the product present in the environment that may be affected?
        - Can dispersal be naturally controlled by barriers in the environment?
        - What is known about the effectiveness of monitoring and mitigation plans?
    - Attributes of the genetic alteration:
        - Does the alteration affect the ability of the product to replicate?
        - What effects could transgene expression or exposure have outside of the target population?
        - What is known about the genetic stability of the altered sequence?
- Assessing the magnitude of potential environmental effects
- Estimates of likelihood of environmental effects
- How to evaluate the overall environmental risk

October 2015, FDA authorised the oncolytic virus vector T-VEC (talimogene laherparepvec, brand name: Imlygic) for the treatment of melanoma lesions in the skin and lymph nodes. An environmental assessment (EA) was prepared pursuant to 21 CFR part 25. The EA provided a quantitative assessment of Imlygic environmental exposure and environmental stability. No significant environmental impacts on the quality of the human environment were identified.

2.2.2 Canada

GM plants

Canada has no specific legislation regarding GM plants. Instead a product-based system is put in place where all plants that have novel traits, be it via genetic modification or mutagenesis or other techniques, or plants that are otherwise new to the Canadian environment, are assessed.
The Canadian Food Inspection Agency (CFIA) defines a plant with a novel trait (PNT) as a new variety of a species that has one or more traits that are novel to that species in Canada. A trait is considered to be novel when it has both of these characteristics:

- it is new to stable, cultivated populations of the plant species in Canada, and
- it has the potential to have an environmental effect.

The Plant Biosafety Office (PBO) evaluates the environmental safety of PNTs under the Plant Protection Act (1990, c. 22) and the Seeds Act (R.S., 1985, c. S-8). Directive 94-08[16] lists which items need to be examined for plants to be permitted for commercialisation. There are five criteria that reflect areas of concern:

- potential of the PNT to become a weed of agriculture or be invasive of natural habitats,
- potential for gene flow to sexually compatible plants whose hybrid offspring may become more weedy or more invasive,
- potential for the PNT to become a plant pest,
- potential impact of the PNT or its gene products on non-target species, including humans,
- potential impact on biodiversity.

Information on experiments and tests that support the ERA must be on the same level as required for the peer-reviewed scientific publications. The characteristics of the PNT are

[15] "gene therapies, vectored vaccines, and related recombinant viral or microbial products"
[16] Directive 94-08 (Dir 94-08) Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits.

compared with its counterpart. The concept of “substantial equivalence” is used. No explicit estimations of likelihood or severity of impacts are made.

Although a case-by-case approach is applied, stacked events are not considered a new case, if the parent plants are already assessed. Consequently there is no requirement for an ERA. Nevertheless, the PBO requires notification of all stacked products before marketing to determine whether additional information is required to assess their safety[17].

Box 4 An example of the areas of concern addressed in a Canadian Directive 94-08 application for the marketing authorisation of a genetically modified crop.

GMO: glyphosate tolerant, yield enhanced soybean event MON-87712-4. This soybean was modified with the gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein from *Agrobacterium* spp. strain CP4 (selectable marker), and the *BBX32* gene from *A. thaliana* which encodes BBX32, a zinc finger protein of the B-Box zinc finger family.
Reference: DD2013-99[18]

Areas of concern addressed:

- Soybean is not likely to become weedy in Canada due to traits such as the lack of seed dormancy and the poor competitive ability of seedlings. Field tests demonstrated that the GM soybean has not changed in that respect. Traits that were evaluated are early stand count, seedling vigour, days to 50% flowering, flower colour, days to 50% end of flowering, days to 50% senescence, days to physiological maturity, plant height, lodging, pod shattering, final stand count, grain moisture, 100 seed weight, and yield.
- The evaluated germination characteristics included percent germinated seed (normal and/or abnormal), percent viable hard seed (an indicator of seed dormancy), percent dead seed, and percent viable firm swollen seed.
- Also, the volunteer potential, susceptibility to biotic and abiotic stressors did not show a change compared to non-modified soybean.
- Soybean may cross with the wild annual species *Glycine soja*. However, this species does not naturally occur in North America. Moreover, soybeans exhibit a high degree of self-fertilisation. No statistically significant differences were detected between the GM and conventional soybean in that respect.
- Soybean is not a plant pest in Canada and the introduced gene for increased yield potential is unrelated to plant pest potential. This means the potential for the plant to harbour new or increased populations of pathogens or pests. Field observations detected no differences in susceptibility to soybean pests.
- Studies revealed that the BBX32 protein amino acid sequence lacks relevant similarities to known allergens and that the protein is not toxic to livestock or NTOs. Composition analyses demonstrated that the levels of key nutrients and anti-nutrients in grain and forage from the GM soybean are comparable to those in conventional soybean varieties. No effects were seen on the nitrogen-fixing soil bacteria associated with soybean cultivation. Some differences were observed in abundance of pest or beneficial arthropod species but they were not consistently detected across collection times or sites. Therefore, these effects are not associated with the novel trait and are not biologically meaningful.
- Effects on biodiversity are not expected: the range of cultivation is not extended. The GM crop has no increased weediness, invasiveness, or plant pest potential, is not different in NTO effects and will not require a change in cultivation practices or crop rotation practices.

[17] http://www.inspection.gc.ca/plants/plants-with-novel-traits/approved-under-review/stacked-traits/eng/1337653008661/1337653513037

[18] http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/eng/1304475469806/1304475550733

Gene therapy

Health Canada is responsible for regulating drugs for use in human clinical trials and commercialisation via the Food and Drugs Act (F&DA) (R.S.C., 1985, c. F-27) and the Food and Drug Regulations (Part C, Division 5) (C.R.C., c. 870). Health Canada reviews clinical trial protocols to assess the protection and safety of the participants.

The Canadian Environmental Protection Act, 1999 (CEPA, 1999) (S.C. 1999, c. 33) requires that all new substances imported into or manufactured in Canada must be assessed for both direct and indirect impacts on human health and the environment. The approach aims to control new substances before they are manufactured or imported into Canada. An assessment needs to be performed to check whether they are potentially toxic and any appropriate or required control measures have to be taken. Only then they may be introduced into Canada.

Since 2001 manufacturers or importers of new substances in products regulated by the F&DA have to notify under the New Substances Notification Regulations (NSNR) (for organisms: SOR/2005-248). Canada's New Substances program is responsible for performing ERAs of new substances (*i.e.*, not listed on the Domestic Substances List), including living organisms and microorganisms that are animate products of biotechnology. Biotechnology is defined in section 3 of Part 6 of CEPA, 1999 as:

> *'the application of science and engineering in the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms'.*

The guidance document 'Preparation of Drug Submissions and Applications in the Common Technical Document (CTD) Format' (Health Canada, 2012) refers in Module 1.5 to the environmental assessment required for new substances in products regulated under the F&DA according to the NSNR of CEPA. This guidance applies to the preparation of all drug submissions and applications for human use, including clinical trial applications.

The type of information that is required under NSNR and the timing of the notification depend on the type of substance, the quantity that will be imported or manufactured, the intended use of the substance and the circumstances associated with its introduction. Micro-organisms that will be used in research and development in a contained facility, under certain conditions (below certain volumes, pathogenicity class) are exempt from the NSNR (subsection 2.3 of the NSNR (Organisms)). However, microorganisms used in gene therapy clinical trials are not exempt, and require notification under Schedule 1 of the NSNR (Organisms) since the organism can be shed into the environment.

The following areas of concern as pronounced in EU legislation may be derived from the data as required by NSNR (Organisms):

- persistence and invasiveness;
- selective advantage or disadvantage;
- potential for gene transfer;
- impact on NTOs (both aquatic and terrestrial plant, invertebrate and vertebrate species);
- effects on human health;
- effects on biogeochemical processes;
- effects on conservation and sustainable use of biological diversity.

Box 5 An example of the areas of concern addressed in a Canadian New Substances Notification application under the NSNR for a clinical trial.

GMO: *Lactococcus lactis* subsp. *cremoris* Strain sAGX0037, a therapeutic drug against inflammatory bowel disease (Crohn's disease). Using homologous recombination sAGX0037 was modified to replace the thymidylate synthase gene (*thyA*) gene of the parental strain *L. lactis* subsp. *cremoris* strain MG1363 with a synthetic expression cassette that encodes for a human interleukin-10 (hIL-10). The GMO produces hIL-10 extracellularly and is dependent on thymine or thymidine supplementation to survive.

Reference: EAU-439[19]

Areas of concern addressed:

- The dependence on thymine or thymidine is a selective disadvantage and will prevent the GMO to be persistent and invasive
- sAGX0037 is deficient of the Tn916 and Tn919 enterococcal conjugative transposons which makes it very difficult to exchange genetic material via horizontal gene transfer.
- A literature study indicated that there is little evidence of any pathogenic potential of *L. lactis* and hIL-10 protein. Pre-clinical studies in mice and monkeys confirmed this.
- *L. lactis* has history of safe use in the food industry and is considered as non-pathogenic to the general population. Safety of the GMO was demonstrated in human clinical trials on patients with Crohn's disease and healthy volunteers.
- Shedding occurs mainly via faeces. Risk to the environment will be negligible as sAGX0037 entering the sanitary sewer system will be inactivated and/or removed by the physical, biological, and/or chemical treatments in place in wastewater treatment plants.
- Several antibiotics are effective in the unlikely event of *L. lactis* sAGX0037 infection in humans.

In addition, the Occupational Health and Safety Acts of the different Canadian provinces and territories that apply to the safety of employees in clinical trials, and the Human Pathogens and Toxins Act (S.C. 2009, c. 24) can be relevant for gene therapy trials in protecting the safety of healthcare workers.

2.2.3 Australia

The Gene Technology Act 2000[20] and the Gene Technology Regulations 2001[21], along with corresponding State legislation, govern the regulation of GMOs in Australia. The Office of the Gene Technology Regulator (OGTR) provides administrative support to the Gene Technology Regulator who is the decision maker for research and commercial applications to work with GMOs. Risk analysis is conducted in accordance with the Act and the regulations.

The same Act and regulations apply to all GMOs, whether for GMPHs or gene therapy products that involve live and viable GMOs. Under the Act it is prohibited to 'deal with' a 'GMO' without authorisation, and 'deal with' and 'GMO' are defined in the Act. Dealings regulated under the Act may pose varying levels of risk to the health and safety of people and the environment, and may therefore require different levels of authorisation and containment.

In the ERA a qualitative approach is taken. The likelihood and consequences of a hazard are estimated using qualitative class values and the approach is described in the Risk Analysis Framework (2013)[22]

For the ERA the Regulator consults with other agencies such as the Food Standards Australia New Zealand (FSANZ) and Australian Pesticides and Veterinary Medicines Authority (APVMA). Some GMOs may also be subject to other regulation because of their use, for example human or veterinary therapeutics, such as live GMO vaccines or gene therapy involving GMOs, would also require assessment and registration by the Therapeutic Goods Administration (TGA) (for human medicines) and APVMA (for veterinary uses).

[19] http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=10CE87F5-1
[20] http://www.comlaw.gov.au/Details/C2014C00587
[21] http://www.comlaw.gov.au/Details/F2011C00732
[22] http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/risk-analysis-framework.

GM plants

According to the Gene Technology Regulations 2001 risk analysis takes into account (Regulations, Part 3, Division 1, 10):

- … any previous assessment by a regulatory authority, in Australia or overseas, in relation to allowing or approving dealings with the GMO; and
- the potential of the GMO concerned to:
  i. be harmful to other organisms; and
  ii. adversely affect any ecosystems; and
  iii. transfer genetic material to another organism; and
  iv. spread, or persist, in the environment; and
  v. have, in comparison to related organisms, an advantage in the environment; and
  vi. be toxic, allergenic or pathogenic to other organisms.

The Regulator must consider both the short term and the long term.

In the guidance document Risk Analysis Framework (OGTR, 2013) further clarifications are provided. Harm may result from:

- Impaired health of organisms due to toxicity or disease
- Reduced quality of biotic components (e.g. reduced biodiversity)
- Reduced quality of abiotic components (e.g. soil, water or air)
- Disruption of ecosystem processes (e.g. altered nutrient levels or fire regimes)

Harm to the environment may have several forms (consequences):

- Harm to the health and safety of people includes:
  - Toxicity or allergenicity
  - Disease
  - Illness or injury
- Harm to the environment includes:
  - Toxicity to desirable (valued) organisms that should be protected
  - Loss of biodiversity, including loss of species diversity or genetic diversity within species
  - Adverse impacts of a new or more serious weed, pest or pathogen
  - Disruption of biotic communities
  - Degradation of the abiotic environment'

The following components are an important part of the information on the GMO needed for the risk assessment:

- Invasiveness (infectivity) *i.e.* the capacity to spread and persist
- Capacity to harm (toxicity, disease, …)
- Capacity for gene transfer (compatible species, horizontal gene transfer)

The Regulator's decisions on authorising a stacked GMO are made on a case-by-case basis as well. Stacked GMOs are commonly authorised through a new licence, but can be authorised by being listed on a specific licence or by inclusion of licence conditions to encompass stacking. If no additional hazards are identified, a stacked GMO may be authorised through variation of a GMO licence as long as it considered all parental lines[23].

Box 6 An example of the areas of concern addressed in an Australian Environmental Risk Assessment for the environmental release ("Dealings involving an Intentional Release") of a genetically modified crop.

GMO: Commercial release of genetically modified (GM) herbicide tolerant and/or insect resistant cotton lines north of latitude 22° South. The ERA considers several events and stacks containing the *Bacillus thuringiensis* toxin genes *cry1Ac* and *cry2Ab*, and the glyphosate tolerance gene *cp4 epsps*.
Reference: DIR-066/2006[24]

[23] http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/gmstacking08-htm
[24] http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/dir066-2006

In the hazard identification process 35 risk scenarios were compiled. While most of them were considered not to give rise to an identified risk (pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process), 6 were further analysed:

Adverse effects to NTOs:

- Direct or indirect ingestion of the Cry1Ac and Cry2Ab proteins by non-target invertebrates.
  The consequences are minor as the proteins are toxic only to lepidopteran insects, which is confirmed by field studies.

Potential of weediness:

- Tolerance to glyphosate due to expression of the *cp4 epsps* gene(s) in the GM cotton plants.
  Cotton is not a weed in Australia and volunteer plants are normally controlled by other herbicides or mechanical means. The selective advantage is offset by susceptibility to the abiotic and biotic factors.
- Reduced lepidopteran herbivory due to expression of the *cry1Ac* and *cry2Ab* genes in the GM cotton plants;
  Cotton is not a weed in Australia and lepidopteran resistance is not the limiting factor on the spread and persistence of cotton. Again, susceptibility to the abiotic and biotic factors nullifies the small advantage of lepidopteran resistance.
- Tolerance to glyphosate and reduced lepidopteran herbivory due to expression of the *cp4 epsps, cry1Ac* and *cry2Ab* genes in combination in the GM cotton plants;
  The herbicide tolerance and insect resistance genes operate through independent, unrelated biochemical mechanisms and there is no evidence of any interaction. The rationale of the items above is therefore equally true for the combined traits.
- Expression of the *cp4 epsps*, and/or *cry1Ac* and *cry2Ab* genes in naturalised *G. hirsutum* or *G. barbadense* cotton plants providing glyphosate tolerance and/or reduced lepidopteran herbivory resulting from vertical gene transfer;
  Cotton is primarily self-pollinating species. The out-crossing frequency is very low. Again, the advantage of herbicide tolerance and insect resistance would be outweighed by other limiting factors. The plants will not establish more easily than non-GM cotton.
- Expression of the cp4 epsps, and/or *cry1Ac* and *cry2Ab* genes in combination with the bar gene (from Liberty Link® Cotton) providing dual herbicide tolerance and reduced lepidopteran herbivory resulting from vertical gene transfer.
  This hazard may arise when the cotton events from this application are grown in proximity of Liberty Link® Cotton. All arguments from the items above hold here as well. Additionally, fields with herbicide tolerant cotton are often sprayed with insecticides also affecting pollinators that will reduce pollen transfer even more.

All statements are supported by studies and literature reviews.

Gene therapy

As the same Act and regulations as for GM plants apply to gene therapy products that involve live and viable GMOs, the same risk assessment process is followed in the ERA conducted by the OGTR.

Box 7 An example of the areas of concern addressed in an Australian Environmental Risk Assessment for the commercial release ("Dealings involving an Intentional Release") of a genetically modified virus for cancer therapy.

GMO: Commercial supply of a tumour-selective genetically modified virus for cancer therapy (Talimogene laherparepvec). The GMO is an attenuated *Herpes simplex virus 1* (HSV-1), modified to selectively replicate in tumours (rapidly dividing cells) and enhance the immune response in treated cancer patients. Two specific viral genes

involved in viral replication and viral antigen presentation have been removed. The *hGM-CSF* gene encoding Granulocyte-Macrophage Colony-Stimulating Factor from humans has been introduced.
Reference: DIR 132[25]

Five risk scenarios were identified.

- Increased disease burden from the GM virus (increase in disease symptoms, or inappropriate immune response) resulting from exposure of staff involved in disposal of the GM virus at clinical sites, leading to viral infection and protein expression.
  Exposure to the GM virus would be minimised by well-established clinical procedures, including the use of Personal Protective Equipment. There is limited shedding of GM. The GM virus is attenuated and does not replicate efficiently in non-dividing cells. The inserted hGM-CSF gene and encoded protein are of human origin and are unlikely to be toxic or cause adverse effects to people. Healthcare personnel who are immunocompromised are excluded from direct or indirect contact with the GM virus. The GM virus is susceptible to anti-viral medication.
- Increased disease burden from the GM virus resulting from exposure of contacts of trial participants (household contacts and animals) to the GM virus, leading to viral infection and protein expression.
  Patients will use clinical dressings to cover the administration site. The GM virus is attenuated and does not replicate efficiently in non-dividing cells. The GM virus is unable to replicate outside the host organism. The viability of GM virus outside the host under environmental conditions is limited. Humans are the only known natural hosts of HSV-1. There is limited shedding of GM virus. The GM virus is susceptible to anti-viral medication.
- Increased disease burden from the GM virus resulting from unintentional release of the GM virus, leading to viral infection and protein expression in other people or animals.
  Transport will be according to appropriate standards for medical products. Storage will be at secure storage or clinical facilities. The GM virus is attenuated, does not replicate efficiently in non-dividing cells and replication only occurs in rapidly dividing cells (tumours). The inserted hGM-CSF gene and encoded protein are of human origin and is unlikely to be toxic or cause adverse effects to people.
- Unintended changes in viral characteristics.
  The tropism of the GM virus is not altered compared to naturally occurring HSV-1. The viral surface would not be altered by the genetic modification.
- Horizontal transfer of genes or genetic elements to other organisms.
  The genetic stability of the GM virus has been confirmed by repeat sequencing of specific areas of the GM virus genome. The GM virus cannot spread effectively into normal tissue, limiting interaction with other viruses. The GM virus is attenuated, does not replicate efficiently in non-dividing cells and replication only occurs in rapidly dividing cells (tumours). GM virus recombination with naturally occurring HSV-1 would not result in a more pathogenic organism than the naturally occurring HSV-1. Recombination with viruses that are not HSV-1 is highly unlikely.

2.2.4 People's Republic of China

GM plants

The Chinese Ministry of Agriculture and its National Biosafety Committee is the competent authority for the biosafety evaluation of new GM crops. The governing law is the 'Agricultural Genetically Modified Organisms Safety Administration Regulations 2001'[26] with three implementing ministerial decrees on safety assessment, import and labelling of GM products.

[25] http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/DIR132
[26] http://www.stee.agri.gov.cn/biosafety/zcfg/gnfg/t20051107_488460.htm

Decree no. 8 concerns the safety evaluation[27]. A qualitative risk classification is used. The GMO is classified into a safety class depending on the safety class of the recipient organisms and the GM activities on that organism.

The Ministry of Agriculture has overall responsibility for the safety assessment with provincial agricultural departments operating on a local scale. In the process of commercialisation applicants first have to apply for a biosafety certificate. This involves 5 steps: research, intermediary experiment, environmental release, productive testing, and biosafety certification. Applicant's Institutional Biosafety Committee internally reviews risk assessments before sending to the provincial administration and finally to the Office of Biosafety Administration under the Ministry of Agriculture. Currently, an amendment is proposed to issue biosafety certificates per ecological zone (USDA FAS-GAIN, 2015). In addition registration is sought of the biotech seed variety at the provincial agricultural department in order to allow for commercial production (variety trials).

The National Biosafety Committee, the scientific advisory committee, developed a guideline for biosafety assessment *i.e.* environmental and food safety[28]. Test are required addressing GMO survival and competiveness, the ecological risk of gene flow, the potential to become a weed, the GMO impact on target and NTOs and biodiversity. Also an anti-nutrient test and a 90-day rat feeding study are to be performed.

China has no policy on stacked events.

Gene therapy

The Drug Administration Law of the People's Republic of China[29] and the Regulations for Implementation of the Drug Administration Law of the People's Republic of China[30] govern all activities involved in medicines, including clinical trials for human gene therapy.

The China Food and Drug Administration (SFDA) is the central agency to oversee the regulation, law enforcement and establishment of national standards concerning food and drugs. It is the competent authority to approve clinical trial studies, register drugs, to review drug manufacturing and inspection, and licensing for drug importation.

For market authorisation approvals are needed for clinical trials phase I, II and III, a New Drug Certificate, a New Drug Registration Certificate, and Drug Good Manufacturing Practice Certificate.

The document 'Points to Consider for Human Gene Therapy and Product Quality Control'[31] outlines the requirements for application of gene therapy clinical study, study protocol format, and requirements for construction of a recombinant DNA and gene delivery system. The document also outlines requirements for establishment and testing of cell banks and engineered strains, manufacturing process, quality controls, and product efficacy and safety tests. While quality control during manufacturing (purity, tests for replicating virus particles etc.), and safety tests in preclinical (tissue targeting, biodistribution, etc.) and clinical studies are essential risk assessment elements, environmental risks are not specifically addressed.

2.3 Comparison between the EU and other approaches

In order to address possible (environmental) impacts related to products of biotechnology, authorities have developed different legal approaches: USA and Canadian authorities apply existing environmental legislation; in the EU, China and Australia GMO-specific legislation including ERA requirements was established.

[27] http://www.stee.agri.gov.cn/biosafety/zcfg/gnfg/t20051107_488581.htm
[28] http://www.stee.agri.gov.cn/biosafety/zhbd/t20070913_782803.htm
[29] http://eng.sfda.gov.cn/WS03/CL0766/61638.html
[30] http://eng.sfda.gov.cn/WS03/CL0767/61640.html
[31] http://www.biopharminternational.com/points-consider-human-gene-therapy-and-product-quality-control-state-food-and-drug-administration-ch

The trigger to conduct an ERA depends largely on the definition of a GMO (GE, PNT …) that is included in the legislation. *E.g*. in the USA a cisgenic plant developed by biolistics using a gene construct entirely derived from plant DNA would not be evaluated by APHIS for cultivation purposes. Stacked events originating from a conventional cross between already approved events require a separate ERA and authorisation in the EU, whereas in the USA and Canada this is usually not the case.

Irrespective these differences, the 6 step comprising ERA methodology is implicitly (Canada, USA) or explicitly (Australia, EU) cited in regulations or guidance documents.

All countries require a case-by-case approach of the ERA. Furthermore, all ERA are based on the comparative assessment with a parental/baseline organism in a specific environment. Both short term and long term effects are considered. All countries stress that scientific soundness in risk assessment is necessary. This convergence of methodology can be linked to the fact that most national and international regulations build on the criteria and principles set out in the Organisation for Economic Cooperation and Development (OECD) Blue Book on "Recombinant DNA Safety Considerations", published in 1986.

Also the areas of concern that are considered in the ERA are very similar in the various jurisdictions and are summarised in Annex 2, Table 1 for GMHPs and in Annex 2, Table 2 for other GMOs. In the tables the areas of concern in EU legislation are taken as a reference (1st column). The information for the other countries is based on legislation, guidance documents or application forms as indicated. Inclusion of an area of concern indicates that a similar concern is raised, although it may differ in the way it is phrased.

For plants, biodiversity in general is a common concern (Annex 2, Table 1). All countries care about the potential for plants to become persistent and invasive and about the potential gene transfer to other plant species. The concern about having a selective advantage or disadvantage is taken up in the EU and Australia. Canada, China and the USA do not mention this explicitly: it may, however, be covered by the item on invasiveness and/or gene transfer.

The impact on target organisms and organisms in the depending food chain is elaborated in the European requirements. NTO effects are clearly an area of concern in all countries. This reflects the concern about biodiversity in general.

Human health, again, is a general concern. All countries are paying attention to the possible immediate and/or delayed effects on animal health and consequences for the feed/food chain. In other countries this may be subject to different legislation.

Biogeochemical processes may be the result of the activity of a diversity of organisms. Only in the EU and in some instances in the USA the assessment of this topic is mentioned, though in other ERAs this may be included as a subsequent effect originating from non-target effects.

Specific cultivation, management and harvesting techniques are taken care of in all investigated countries but China.

Other concerns that arise are the potential for the GMHP to become a plant pest or to have altered disease and pest susceptibilities. An ERA for market introduction in the USA also requires assessing the effects on the abiotic environment like land, soil, air, climate change, and surface and groundwater quality. Climate change effects, like changes in greenhouse gas production, in the EU may be covered by cultivation, management and harvesting techniques.

For gene therapy trials and products (Annex 2, Table 2) all countries assess the potential for GMOs to become persistent and invasive, for selective advantage or disadvantages, and on the potential gene transfer to other species. In all countries, the patient is considered the target organism and his/her safety, being covered by other legislation, is not subject to the ERA. Effects on NTOs and the human health other than the patient's health is a common area of concern. Depending on the vector type the following elements are deemed less relevant in gene therapy applications:

- effects on animals and consequences for the feed/food chain for vectors derived from human pathogens;
- effects on biogeochemical processes for viral vectors (as opposed to bacterial vectors);
- effect of change in management techniques (gene therapies often open completely new treatment options with no relevant comparator).

Some EU countries assess clinical trials as contained use applications, or as a mixture of both contained use and deliberate release (Perseus, 2006). In these cases potential risks are mitigated by physical containment. Nevertheless, for market approval dossiers, data still need to be collected to address the areas of concern as indicated in Annex II of Directive 2001/18. Likewise, in the USA the ERA data requirements are postponed to the later phase clinical trials with gene therapy products.

2.4 Other possible areas of concern

The EU regulatory framework has been adverted to be very stringent and comprehensive. Nevertheless, the topics covered in other countries were lined up with the EU indications in order to identify areas of concern that may not yet be covered in EU legislation. Overall it can be concluded that no topics were identified that are not covered in the EU regulatory framework.

For plants the following concerns are explicitly covered in the USA:
- potential for the GM plant to become a plant pest;
- potential to have altered disease and pest susceptibilities;
- adverse effects on the abiotic environment like land, soil, air, climate change, surface and groundwater quality.

While they seem relatively unique, it can be argued that these points are covered in other European areas of concern or by other EU legislation (*e.g.* relating to plant varieties, plant protection products, etc.). For GMOs other than plants no differences were found between the studied countries.

At its fourth meeting, the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety established an open-ended online forum on specific aspects on risk assessment. In this respect the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management organised different discussion groups and real time online conferences, one was specifically addressing topics for which participants felt that guidance materials on risk assessment had to be developed. Annex V of the final report (UNEP/CBD/BS/AHTEG-RA&RM/2/5[32]) indicates priorities for the development of guidance. While these topics address important methodological aspects, they do not identify additional areas of concern.

It must be reminded that the areas of concern are only an operational tool to circumvent the basic lack of identification of protection goals and workable SPGs. EFSA has published guidance documents in relation to SPGs for the ERA of other products (*e.g.* plant protection products). Mid 2015 a consultation was organised on a draft guidance document[33] on defining protection goals for ERA in relation to biodiversity and ecosystem services which also covers GMOs as potential stressors.

Further improvement of the ERA can only be justified on explicit recognition of policy driven objectives that can be applied independently of the product subjected to the ERA. The FP7 EU project AMIGA (Assessing and Monitoring the Impacts of Genetically modified plants (GMPs) on Agro-ecosystems)[34] aims amongst other targets at translating regional protection goals in measurable assessment endpoints. This is part of problem formulation and may contribute in defining specific areas of concern. Problem formulation as the first step in the ERA identifies the characteristics of the GMO capable of causing potential adverse effects. In this step hypotheses will have to be set and translated into a researchable question. To this end measurable

[32] https://www.cbd.int/doc/meetings/bs/bsrarm-02/official/bsrarm-02-05-en.pdf
[33] EFSA, Question No EFSA-Q-2013-00901
[34] http://www.amigaproject.eu/; end of the project tentatively end of November 2015

assessment endpoints need to be determined. The general areas of concern may then be detailed with specific areas of concern.

This section reviewed areas of concern as applied in ERA of GMHP and gene therapy. The authors acknowledge that stakeholders and the public at large voice additional concerns that influence the societal debate on these developments. Trying to address them in an ERA will most likely not result in an adequate answer. *E.g*. a discussion on independency and reliability of data acquisition cannot be solved by more data, but rather by strengthening the confidence in the controls on the provided information. In gene therapy the general public view focusses on patient safety and ethical issues, rather than environmental effects.
While these concerns may reflect important policy options and methodological challenges, they may not fit in the framework of an ERA of a specific trial or product. A different approach should be available to identify what can be addressed through the ERA and how those aspects not fitting can be handled adequately.

3 Information relevant for ERA

When the European legal framework for the ERA of GMOs was introduced 25 years ago, only a limited amount of information was available on the behaviour and potential impact of the GMOs. Only a few GMHPs and GM micro-organisms had been the subject of limited field trials. Since then, different research programmes have looked at diverse aspects relevant to the ERA. At the same time GMOs enabled ecological research that beforehand had been difficult to realise. They also provide a better understanding on environmental interactions of both non-genetically modified organisms and their GM equivalents. Finally, concerns over the potential effect of GMOs motivated funding of research on topics that had received limited interest. In line with the precautionary principle, further research is one of the ways to address uncertainty.

As part of this exploratory study, an overview is given of different initiatives. Realizing that it is impossible to be comprehensive, it must be reminded that the main goal of this report is to identify areas in which our understanding has evolved to a level that provides confidence for conclusions in the ERA.

3.1 Plants

3.1.1 Reports from governmental bodies

Documents have been issued by governmental bodies such as authorities and advisory committees. They are particularly important as they reflect the position of that body on a specific issue. They already serve as a "generic" evaluation as they are produced with a clear objective. Usually they serve as such as reference for the ERA.

Biology documents

Biology documents were developed to provide a basis for comparing the behaviour of GM crops with that of the non-modified counterparts. Internationally, OECD in the frame of their 'Work on Harmonisation of Regulatory Oversight in Biotechnology' published biology documents for a large variety of plants and traits[35]. These consensus documents comprise technical information for use during the regulatory assessment of products of biotechnology and are intended to be mutually recognised among OECD Member countries. The documents are updated to take into account new knowledge on the topic.

This approach is further elaborated by *e.g.* the Plant Biosafety Office of the Canadian Food Inspection Agency. They published biology documents[36] as companion documents to the guidance regarding the submission of an application for the authorisation of the unconfined release of a plant with a novel trait (Directive 94-08 (Dir94-08), Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits). Specifically, this information is used to determine whether there are significantly different/altered interactions with other organisms resulting from a PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or otherwise interacts differently than their counterpart in the environment.

Similarly, the Australian Office of the Gene Technology Regulator prepared biology documents[37] to inform the Risk Assessment and Risk Management Plans (RARMPs) related to dealings involving intentional release (DIRs).

Table 1 summarises the available biology documents.

[35] http://www.oecd.org/science/biotrack/consensusdocumentsfortheworkonharmonisationofregulatoryoversightinbiotechnology.htm

[36] http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/eng/1330723572623/1330723704097

[37] http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/biology-documents-1

Table 1 Biology documents issued by the OECD and the Australian and Canadian authorities

Species		OECD	Australia	Canada
Alfalfa	*Medicago sativa*			X
Apple	*Malus domestica*			X
Banana	*Musa spp.*	X		
Barley	*Hordeum vulgare*		X	
Black Spruce	*Picea mariana*	X		
Brassica crops	*Brassica spp.*	X		
Brown Mustard	*Brassica juncea*			X
Camelina	*Camelina sativa*			X
Carnation	*Dianthus caryophyllus*		X	
Cassava	*Manihot esculenta*	X		
Cotton	*Gossypium hirsutum*	X	X	
Douglas-fir	*Pseudotsuga menziesii*	X		
Eastern white pine	*Pinus strobus*	X		
Eucalyptus	*Eucalyptus* spp.	X		
European white birch	*Betula pendula*	X		
Flax	*Linum usitatissimum*			X
Italian ryegrass	*Lolium multiflorum*		X	
Jack pine	*Pinus banksiana*	X		
Larch	*Larix* spp.	X		
Lentil	*Lens culinaris*			
Lodgepole Pine	*Pinus contorta*	X		
Lupine	*Lupinus*		X	
Maize	*Zea mays*	X	X	X
Mustard – Polish canola	*Brassica rapa*			X
Norway spruce	*Picea abies*	X		
Oilseed rape	*Brassica napus*	X	X	X
Papaya	*Carica papaya*	X	X	
Pepper	*Capsicum annuum*	X		
Perennial ryegrass	*Lolium perenne*		X	
Tall fescue	*Lolium arundinaceum*		X	
Pineapple	*Ananas comosus* var. *comosus*		X	
Poplar	*Populus* spp.	X		
Potato	*Solanum tuberosum*	X		X
Rice	*Oryza sativa*	X	X	
Rose	*Rosa x hybrida*		X	
Safflower	*Carthamus tinctorius*		X	
Sitka spruce	*Picea sitchensis*	X		
Soybean	*Glycine max*	X		X
Squashes, pumpkins, zucchinis and gourds	*Cucurbita* spp.	X		
Stone fruits	*Prunus* spp.	X		
Sugarbeet	*Beta vulgaris*	X		X
Sugarcane	*Saccharum officinalis*	X	X	
Sunflower	*Helianthus annuus*	X		X
Torenia	*Torenia x hybrida*		X	
Wheat	*Triticum aestivum*	X	X	X
Western white pine	*Pinus monticola*	X		
White clover	*Trifolium repens*		X	
White spruce	*Picea glauca*	X		

Novel Trait documents

OECD also compiled documents describing specific traits irrespective of the crop plant. Information is given on the genes, the structure and properties of the proteins they encode, mechanisms of action, use in plants, toxicity and exposure data, and assessment methods.

- Consensus Document on General Information concerning the Biosafety of Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection (1996)
- Consensus Document on General Information Concerning the Genes and Their Enzymes that Confer Tolerance to Glyphosate Herbicide (1999)
- Consensus Document on General Information Concerning the Genes and Their Enzymes that Confer Tolerance to Phosphinothricin Herbicide (1999)
- Herbicide Biochemistry, Herbicide Metabolism and the Residues in Glufosinate-Ammonium (Phosphinothricin)-Tolerant Transgenic Plants (2002)
- Consensus Document on the Safety Information on Transgenic Plants Expressing *Bacillus thuringiensis* – Derived Insect Control Protein (2007)

The consolidated presentation of the joint Scientific Opinion of the GMO Panel and the Panel on Biological Hazards (BIOHAZ) on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants"[38] elaborates on the potential environmental hazards of two selection markers used in plant biotechnology: ***aph(3')-IIa*** (***nptII***; kanamycin/neomycin resistance) and ***ant-(3")-Ia*** (***aadA***; streptomycin/spectinomycin resistance). An overview of relevant scientific literature is given and a qualitative risk assessment is provided.

GM Food – Feed documents

OECD also issued consensus documents on key food and feed nutrients, anti-nutrients, toxicants and allergens for several crops. This information is compared with the composition of the GM crop in order to investigate potential risks for humans and animals coming into contact with the GM crop. Workers on farms or in the processing industry may accidently ingest or inhale dust or have skin contact. Animals that normally feed on the crop in the field may be affected as well.

Documents are available for alfalfa, cassava, cotton, barley, maize, oilseed rape, papaya, potato, rice, sorghum, soybean, sugarbeet, sugarcane, sunflower, sweet potato, tomato and wheat[39].

3.1.2 Research commissioned by a governmental body

Whereas the previous section addressed information issued by a governmental body with a clear aim to support the ERA, research is commissioned on specific topics of interest. In such case, the outcome may provide important insights, but is not necessarily reflecting the position of the funding body.

EFSA

EFSA commissioned studies that address specific topics. The studies may collect background data or sources of data and may provide tools and methodologies that may be useful in conducting ERAs. Also, as explained before, research related to food and feed safety might be relevant to the ERA especially for the areas of concern of human and animal health.

In some cases the research did lead to a statement of the funding body: *e.g*. EFSA commissioned a study on the statistical methods and data requirements to support Post-Market Environmental Monitoring (PMEM) of agro ecosystems (OC/EFSA/SAS/2012/02). PMEM is an additional source of information regarding the environmental impact of crops that are already

[38] http://www.efsa.europa.eu/en/efsajournal/pub/1108.htm
[39] http://www.oecd.org/science/biotrack/consensusdocumentsfortheworkonthesafetyofnovelfoodsandfeeds.htm

commercialised. The PMEM report addresses Case-Specific Monitoring (CSM) for those cases where in the pre-commercial ERA potential adverse effects of GMOs or their use were identified, and General Surveillance (GS) that is meant to detect unanticipated adverse effects of the GM crop, if any.

EFSA also organised colloquia and stakeholder consultations on specific topics to support its risk assessment work. Through presentations and discussions the views of experts and stakeholders is collected and are reflected in the event reports.

A list of relevant studies and events is provided in see Annex 3, Table 1.

The Netherlands

The Netherlands Ministry responsible for Environment ordered RIVM to perform a study on the suitability of certain environmental monitoring networks to support the PMEM with data (RIVM Report 601040001/2012). Another study evaluates whether tests for chemical pesticides may be used to test the proteins that are encoded by the introduced gene(s) (RIVM Report 601787002).

COGEM commissioned a variety of reports dealing with the impact of GMOs on the environment. They tackle several areas of concern: gene transfer (CGM 2005-02 on gene transfer to bacteria), effect on NTOs (CGM 2012-06 on statistical models; CGM 2014-02 and CGM 2014-05 on *Bt* toxins, their mode of action and potential interaction; CGM 2014-06 on field trials to study NTO effects; and CGM 2015-02 on effects on aquatic organisms), human and animal health (CGM 2013-04 on the contribution of metabolomics in ERA), biogeochemical processes (CGM 2013-03 on guidelines to study interactions with soil organisms), and changes in crop management (CGM 2012-09 on potential changes for maize, sugarbeet and potato).
Again, some PMEM related studies are included (CGM 2010-08 on USA experience, CGM/130402-01 on PMEM in import dossiers).

COGEM also published 2 event reports, both addressing the impact of Bt proteins on NTOs.

A list and short summary is presented in Annex 3, Table 2.

Other EU Member States

Also in other countries several initiatives were launched to examine the environmental impact of GMOs. The EU funded PreSto GMO ERA-Net (see 3.1.3) inventoried research in the area of GMO effects at Member State level, specifically regarding the health, environmental and techno-economic effects of GMOs in Europe.

An example for the UK are the Farm Scale Evaluations (FSE)[40] of GM herbicide tolerant crops commissioned by the UK government Department for Environment, Food & Rural Affairs (DEFRA). The aim was to verify the concern that further intensification of farming with herbicide tolerant GM crops would reduce arable biodiversity to the point where species would disappear and essential functions of the habitat would be impaired. In the 4-year study a comparison was made with weed control as conventionally used with non-GM crops for 4 GM-crops (maize, spring and winter oilseed rape and sugarbeet).

In 2005 the report 'Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance' ordered by the Austrian Bundesministerium für Gesundheit was published (Dolezel *et al.*, 2005). The literature study gathered information on ecological effects of maize mostly containing the Cry1Ab toxin, and a few other Bt toxins. The authors identify weaknesses in laboratory studies and point to the difficulty of translating laboratory results on NTOs to field situations. According to the studies available at that time major effects on NTO abundance due to Bt maize cultivation seemed to be rather unlikely. Also, the ecological effects of herbicide tolerant maize were summarised.

[40] http://webarchive.nationalarchives.gov.uk/20080306073937/http://www.defra.gov.uk/environment/gm/fse/

The Bundesministerium für Gesundheit furthermore funded the TOXPROT project (Toxic Properties of Novel Proteins in GMOs) that lasted from 2005 till 2007. The study reviews the applied approaches in toxicity and ecotoxicity assessment of novel proteins and identifies and discusses possible weaknesses and limitations (Spök *et al*., 2008). Starting with the available general knowledge on protein toxicity the study continues with specific knowledge on proteins expressed by GM plants, the assessment methodologies and evidence of toxicity for humans and ecotoxicity. Information is sought in guidance documents, regulatory dossiers and literature. The supplemental tables inventory, amongst others, the test methods for various proteins in GM plants as presented in commercial applications in the EU and the USA, and the proteins that are studied in NTOs as part of regulatory dossiers for food and feed in the EU, as available at the time of the project.

3.1.3 Government funded research projects

EU

The EU has been funding research and innovation establishing subsequent Framework Programmes (FP) that call for co-operated research of a variety of disciplines to tackle a diversity of public developments.

Since FP1 several projects were started that performed research related to the ERA of GMOs. Projects were targeted either to the development of methods for analysis of certain phenomena or to study specific areas of concern using certain crop species.

Initially the focus was on gene transfer and its consequences for the environment (Annex 3, Table 3). Gene transfer was studied among plants and between micro-organisms and plants (12 projects throughout all FPs). Potato, alfalfa, oilseed rape, wheat, rice, maize, lettuce, chicory and sugarbeet were the studied crop species.

From the mid-nineties also other GMO related concerns were addressed, such as effects on NTOs (11 projects), assessment of virus resistant plants and stability of gene expression (2 projects). Other topics that were examined later included effects on soil microbiota (4 projects), the development of genetic tools for detecting Bt resistance genes in target organisms (1 project), co-existence (1 project), making crops disease and pest resistant (2 projects), the assessment of potato in the Central Andes and Bt transgenic cotton in China, and on the effect of antibiotic resistance genes (2 projects).

The PreSto GMO ERA-Net project[41] was intended to lay the groundwork for transnational research on health, environmental and techno-economic impacts of GMOs by:

- Identifying gaps in the existing knowledge relevant for risk-benefit considerations of current and future GMO applications.
- Significantly improving the alignment of ongoing and future research programmes of the individual Member States, in order to avoid duplication of work, to leverage complementarities, and to enhance cooperation between scientists from all over Europe to create an internationally recognizable critical mass in terms of expertise and capacities.
- Promoting the accessibility of existing scientific information to interested stakeholders, regulators and end-users.
- Supporting an open communication and dialogue on all societally relevant issues associated with GMOs that takes into account both benefits and risks. This will allow making an informed choice about whether and how biotechnologies can be used to deliver solutions to the current and future challenges in agriculture and other areas.

One aspect of PreSto GMO ERA-Net was obtaining a comprehensive overview of research in the area of GMO effects at Member State level and internationally. Analysing 320 GMO projects in Europe supports the conclusion that GM plants was the category most widely assessed. Considerably fewer projects studied the effects of other organisms such as GM microorganisms and GM animals. Analysis of the scientific literature related to three major cases of GMOs (Bt maize, GM herbicide tolerant plants, and GM growth enhanced Atlantic salmon) was performed

[41] http://www.presto-gmo-era-net.eu/home

in order to map the research activities to identify the key actors in GM research in Europe. Most of the publications explored the effects of Bt maize and GM herbicide tolerant plants on the environment and impacts on the preservation of biodiversity, mainly on non-target organisms. The information gathered is fed into a publicly available database.

One on-going project aims at producing scientific data related to the possible environmental and economic impacts of cultivation of GM crops in general (AMIGA[42]). At the same time, the GMO Risk Assessment and Communication of Evidence (GRACE[43]) project serves 2 related aspects: One is to collect information on environmental and socio-economic aspects of GM crop cultivation through systematic reviews of the available literature; another aspect is the evaluation of animal feeding trials as tests for food and feed safety. Results of both projects are becoming available since November 2015, the end of the projects at the time of conclusion of this report, the following conclusions and recommendations[44] from the GRACE project were available:

- Systematic reviews are relatively new to the field of GMOs. They offer the advantage of reviewing literature according to a well-defined protocol. Important elements are the transparency, reproducibility and the assessment of the quality of the publications. The limitations are that they can be demanding on resources such as time, money, and manpower, and only tackle a specific research question at a time. A potential major limitation restricting the use of systematic reviews is the availability of primary research data, as most of the information to be provided in the approval process for GMOs is focused on a specific event.
- While detailed conclusions drawn from GRACE systematic reviews and evidence maps will be provided in the respective publications, GRACE reviews confirm the conclusions of previous risk assessments of Bt and herbicide tolerant (HT) crops with regard to field impacts on the evaluated groups of organisms. They provide complimentary scientific information that may inform risk assessors and managers, and those involved in environmental monitoring and integrated pest management. They provide weight of evidence information that may inform those making policy and decisions.
- Evidence maps can inform both risk assessment and risk management communities. The drafted evidence maps on the four health-related topics (toxicity of newly expressed proteins and whole foods/feeds, allergenicity, composition) showed that publications straddle a wide range of crops, newly introduced traits, experimental animal species and other experimental models and parameters employed.
- The references retrieved through the search actions but de-selected during further selection and extraction, may also provide interesting insights, such as the observation that a high number of studies have been published on the production and characteristics of oral vaccines and other pharmaceuticals in GM crops (used as “plant factories”), and the seemingly increasing number of publications by Chinese and other Asian authors in recent years (*e.g.*, in non-English literature).
- Evidence gaps and areas for further research have been identified in terms of geographical focus and research fields (e.g. supply chain, environmental economics, food security, distribution impact of trade-related measures among actors).
- Preliminary conclusions revealed that the introduction of GM crops does matter in terms of aggregate welfare change and that there are mixed results on trade-related impacts of GM crops, some of which are in line with previous empirical findings.

Finally, the gathered information of both GRACE and PreSto GMO ERA-Net will be available at the publicly available “Central Access Database for Impact Assessment of Crop Genetic Improvement Technologies” CADIMA[45]. The open-access database is a non-profit internet portal aiming to increase the transparency and traceability of information being associated with the impact/risk assessment of plant genetic improvement technologies. It provides a comprehensive overview about background information related to the risk assessment process

[42] www.amigaproject.eu/
[43] http://www.grace-fp7.eu/
[44] GRACE Conclusions and recommendations on animal feeding trials and alternative approaches and on the use of systematic reviews and evidence maps for GMO impact assessment http://www.grace-fp7.eu/sites/default/files/GRACE_Conclusions%20&Recommendations.pdf
[45] http://www.cadima.info/

per se, supports the conduct of so called systematic reviews and evidence maps and grants access to raw data generated by associated research activities.

The Netherlands

The Netherlands Organisation for Scientific Research (NWO) funded the ERGO (Ecology Regarding Genetically Modified Organisms)[46] programme that lasted from 2006 till 2012.
The ERGO programme was to provide tools to improve the environmental risk assessment of new GMOs. Twenty-five projects were honoured mainly targeted to basic functions of the ecosystem. The final report[47] summarises that the normal variation in micro-organism communities is even higher than assumed before. Establishing a 'normal operating range' of soil ecosystems may be of help in the ERA of new GM crops.
Topics were:

- multitrophic interactions
- outcrossing and introgression with wild relatives
- functioning of soil ecosystems

A list of projects, theses and publications is available in Annex 3 Table 4.

3.1.4 Consortium sponsored research

BRIGHT (Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance, 1999-) a UK government/industry consortium project sponsored by DEFRA, Scottish Executive Environment and Rural Affairs Department (SEERAD), Home Grown Cereals Authority (HGCA, rebranded as AHDB Cereals & Oilseeds), British Beet Research Organisation (BBRO), Aventis, Agrovista, Monsanto was to analyse the agricultural implications arising from the use of herbicide tolerant crops, including genetically modified varieties, in arable rotations. Herbicide tolerant winter oilseed rape and sugar beet were grown in four year arable rotations with cereals and other crops to study weed control. The final project report[48] concluded that no significant decreases in botanical (species) diversity were observed between the treatments. It was also confirmed that the herbicide tolerant winter oilseed rape cultivars were no more persistent than the conventional cultivar.

3.1.5 Information from applications

Research & Development trials

Worldwide thousands of field trials have been carried out testing a multitude of GM crops and traits. The European JRC database on experimental releases (Deliberate release into the environment of GMOs for any other purposes than placing on the market[49]) lists field trial applications including a short summary of the 'Environmental Impact and Risk Management'. In some cases also the outcome of the trials is publically available.

COGEM commissioned a report inventorying field trial data all over the world: Survey of Field trials with Genetically Modified Plants - Global trends and developments (CGM 2014-04). This report provided an insight in future developments, *i.e.* crop-trait combinations that might enter the market in the coming years. The most trialed crop is maize followed by soybean, oilseed rape and cotton. Herbicide tolerance and biotic stress protection (*e.g*. insect resistance) remain the dominant trait added by abiotic stress tolerances (mainly drought tolerance and nitrogen use efficiency). Other traits are modifications to increase yield and to alter oil, protein and starch composition.

[46] http://www.nwo.nl/en/research-and-results/programmes/Ecology+Regarding+Genetically+Modified+Organisms
[47] http://www.nwo.nl/actueel/nieuws/2014/alw/afsluitende-publicatie-programma-ecologie-rond-genetisch-gemodificeerde-organismen-ergo.html
[48] http://cereals.ahdb.org.uk/publications/2004/november/29/botanical-and-rotational-implications-of-genetically-modified-herbicide-tolerance-in-winter-oilseed-rape-and-sugar-beet-(bright-project).aspx
[49] http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx

Commercial release

In a dossier to apply for market introduction of a GM plant information is provided to be able to perform an ERA. The requirements are listed in Annexe III B of Directive 2001/18/EC. A schematic overview provided in Annex 4, Table 1 lists the required data for the distinct areas of concern for GM plants. Application dossiers can be consulted, while the summary notifications (Summary Notification Information Format, SNIF) are available on the GMO compass website[50].

Worldwide 380 events and stacks are authorised of which 354 for cultivation. Since the first plantings in 1996, a cumulative hectarage of more than 1.8 billion hectares have been successfully cultivated (Clive J., 2014). The final ERA reports (opinions, assessment reports, etc.) of the competent authorities are posted on the authority's websites and the Biosafety Clearing House (Annex 5, Table 1). Table 2 provides an overview of the detailed information listed in Annex 5, Table 2.

The most prevalent trait is herbicide tolerance (262 events and stacks) and insect resistance (198 events and stacks). Virus resistance is the other biotic stress resistance trait (24 events; bean, papaya, plum, potato, squash, sweet pepper and tomato). In the category of product quality 76 events and stacks are listed (altered flower colour, altered lignin, delayed ripening, increased shelf life, modified alpha amylase, modified amino acid production, modified oil/fatty acid production, modified starch production, phytase production, thermostable alpha-amylase). Four drought tolerant maize events and stacks and 3 drought tolerant sugarcane events are listed in the category of abiotic stress tolerance.

Post-release (monitoring) data are valuable as well for future risk assessment. This is information collected by the applicants after the introduction into the market of the GM crop. In the EU the PMEM is mandatory for GM crops authorised for cultivation and, where appropriate, a Post-Market Monitoring (PMM) for products authorised for food, feed, import and processing. Case-Specific Monitoring is required when risks or gaps in scientific knowledge or significant levels of critical uncertainty are detected in the ERA. Additional research may be needed to address these items. The general surveillance part of PMEM is intended to detect unanticipated environmental hazards. It generally consists of 3 elements (EFSA, 2011b). The monitoring of the GM crop and its cultivation sites is usually performed using farmer questionnaires. Secondly, the impact of GM crop cultivation in the wider environment is measured using data collected from a range of existing monitoring networks which observe changes in biota and production practices. And thirdly, the literature is reviewed for all new scientific, technical and other information relating to the GM crop, as well as of similar crop/trait combinations. A list of PMEM reports for cultivated GM crops and the corresponding EFSA opinions is presented in Annex 5, Table 3.

Outside the EU monitoring obligations exist in Brazil. A yearly report has to be submitted for each of the first five years of commercial cultivation (Normative Resolution No. 5, 2008). These reports are confidential. A substantial effort was seen for a glyphosate tolerant soybean authorised in 2005 (GTS 40-3-2). Five years of comparative assessment in representative soybean growing areas confirmed the ERA (Oral communication M. McLean at the EuropaBio Workshop "Monitoring cultivation of genetically modified crops", 2010). Other countries require the applicants to report adverse effects, often via a stewardship plan (Canada, India, and USA). No adverse environmental impacts identified. None of the efforts identified adverse environmental impacts.

[50] http://www.gmo-compass.org/eng/gmo/db/

Table 2 Summary of commercially authorised crops (total).
Number of events and stacks with certain traits: AS: Abiotic stress tolerance, PB: Plant biology, HT: Herbicide tolerance, IR: Insect resistance, VR: Virus resistance, PQ: Product quality, PS: Product systems, MG: Marker genes.

	Agronomic properties			Biotic stress resistance		Product specifications			Total
Crop (Species)	**AS**	**PB**	**HT**	**IR**	**VR**	**PQ**	**PS**	**MG**	
Alfalfa (*Medicago sativa)*			4			2		2	5
Apple (*Malus domestica)*						2		2	2
Bean (*Phaseolus vulgaris)*					1				1
Carnation (*Dianthus caryophillus)*			19			19			19
Chicory (*Cichorium intybus)*		3	3					3	3
Cotton (*Gossypium hirsutum)*			41	42				32	56
Creeping Bentgrass (*Agrostis stolonifera)*			1						1
Eggplant (*Solanum melongena)*				1				1	1
Eucalyptus (*Eucalyptus sp.)*		1						1	1
Flax (*Linum usitatissumum)*			1					1	1
Maize (*Zea mays)*	4	6	125	115		12		72	140
Melon (*Cucumis melo)*						2		2	2
Oilseed rape (*Brassica napus)*		16	29			7		14	36
Papaya (*Carica papaya)*					4			3	4
Petunia (*Petunia hybrida)*						1			1
Plum (*Prunus domestica)*					1			1	1
Poplar (*Populus nigra)*				2				2	2
Potato (*Solanum tuberosum)*			4	30	14	12		26	42
Rice (*Oryza sativa)*			3	3			1	2	7
Rose (*Rosa hybrida)*						2			2
Soybean (*Glycine max)*			27	4		7		2	30
Squash (*Cucurbita pepo)*					2			1	2
Sugar Beet (*Beta vulgaris)*			3					2	3
Sugarcane (*Saccharum sp)*	3							1	3
Sweet pepper (*Capsicum annuum)*					1				1
Tobacco (*Nicotiana tabacum)*			1			1		1	2
Tomato (*Solanum lycopersicum)*				1	1	9		8	11
Wheat (*Triticum aestivum)*			1						1
Total	7	26	262	198	24	76	1	179	380

3.1.6 Scientific reports/publications

Different aspects of and relevant to GM plant ERA have been published in scientific literature. The International Centre for Genetic Engineering and Biotechnology (ICGEB) established a specific Biosafety Bibliographic Database[51] originating from CAB ABSTRACT database published by CABI UK. The database contains the full reference and abstract of scientific studies on biosafety and risk assessment in biotechnology published since 1990. The database is updated monthly. Table 3 gives the results of the number of individual publications mentioned in the database when searching for a selected set of search terms.

Table 3 Number of 'hits' resulting from searching the ICGEB Biosafety Bibliographic Database (search performed in August 2015)

Search term	Number of hits in a general search	Number of hits relating specifically to environmental risks
Traits		
Bt toxin	3236	427
Herbicide	1454	650
Abiotic	137	58
Drought	106	36
Crops		
Maize	2012	664
Cotton	1240	438
Oilseed rape	852	273
Rice	788	279
Potato	528	203
Soybean	464	79
Sugarbeet	217	87
Areas of concern		
Animal health	2405	78
Human health	1925	156
Pollen flow	1888	449
Selective advantage	737	41
Non-target	549	410
Persistence	355	226
Invasiveness	81	61
Biogeochemical	11	5
Meta-analysis	16	9

Some of the publications reporting on meta-analysis of certain aspects relevant to ERA are briefly discussed below in Box 8. Most of them concern effects on NTA.

Box 8 Overview of meta-analysis relevant for ERA of GM crops

Comas, C., Lumbierres, B., Pons, X., Albajes, R., 2014, No effects of *Bacillus thuringiensis* maize on nontarget organisms in the field in southern Europe: a meta-analysis of 26 arthropod taxa. Transgenic Research, 23, (1), p.135-143.

The article reports on 13 field trials that were conducted in Spain to measure the effects of Bt maize on a broad range of arthropod taxa. By combining several trials in a meta-analysis the statistical power may be improved whereas single trials rarely

[51] http://biosafety.icgeb.org/activities/bibliosafety

have the statistical power to detect low effect sizes. The report concludes that Bt maize has no effect on the most common herbivore, predatory and parasitoid arthropods found in the maize ecosystems of southern Europe.

Knox, O., Hall, C., McVittie, A., Walker, R., Knight, B., 2013, A systematic review of the environmental impacts of GM crop cultivation as reported from 2006 to 2011. Food and Nutrition Sciences, 4, (6A), p.28-44.
A meta-analysis was conducted on 28 articles about environmental impacts that GM crop cultivation may have. The authors state: '*The conclusion of this study is that GM adoption has had an impact on its environment, but that the underlying cause of this impact is not due to the genetic modification and the effect on the environment is generally seen as not being adverse*.'

Nakasu, E.Y.T., Dias, S.C., Pires, C.S.S., Andow, D.A., Paula, D.P., Togni, P.H.B., Macedo, T.R., Sujii, E.R., Sa, M.F.G. de, Fontes, E.M.G., de Sa, M.F.G., 2013, Bitrophic toxicity of Cry1Ac to *Cycloneda sanguinea*, a predator in Brazilian cotton. Entomologia Experimentalis et Applicata, 148, (2), p.105-115.
Cycloneda sanguinea is one of the main predators of non-target pests in Brazilian cotton. Feeding experiment with aphids sprayed with a Cry1Ac solution resulted in data on pupal survival, development time, aphid consumption, and adult longevity, and net replacement rate. No significant differences due to Cry1Ac were observed. A meta-analysis was performed to evaluate independence of responses. This analysis suggested that all effects were not different from zero and no cumulative effects could be detected.

Albajes, R., Lumbierres, B., Madeira, F., Comas, C., Ardanuy, A., Lee, M.S., Iglesias, S., Comas, J., Perez-Hedo, M., Lopez, C., Eizaguirre, M., Pons, X., Romeis, J., Meissle, M., 2013, Field trials for assessing risks of GM maize on non-target arthropods in Europe: the Spanish experience. IOBC/WPRS BulletinProceedings of the IOBC/WPRS Working Group 'GMOs in Integrated Plant Production', Berlin, Germany, 3-5 June 2013. 97, p.1-8.
Twenty field trials conducted in Spain from 1998 to 2010 to assess risks of insect resistant plants based on expression of a toxin of *Bacillus thuringiensis* (Bt) and herbicide-tolerant (HT) maize were statistically analysed for abundance of herbivore, predator, parasitoid and detritivore arthropods with conventional ANOVA and with meta-analysis. The meta-analysis approach allowed for drastically improving statistical power and confirmed former conclusions that no effects of the expression of different Bt toxins on NTAs in maize were found in the field.

Marvier M., 2011, Using meta-analysis to inform risk assessment and risk management. Journal für Verbraucherschutz und Lebensmittelsicherheit 6(1) Supplement, p. 113-118.
The publication states that meta-analysis has a great potential to inform GMO ERA, providing quantitative syntheses of the benefits, risks, and information gaps. Meta-analysis is a set of statistical techniques that generates a quantitative summary of the size of the effect caused by a treatment.

Lovei, G.L., Andow, D.A., Arpaia, S., 2009, Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. Environmental Entomology, 38, (2), p.293-306.
The authors reviewed peer-reviewed literature about the impact of GM plants on arthropod natural enemies in laboratory experiments and found a continued bias toward studies on a few predator species, especially the green lacewing, *Chrysoperla cornea*. This species may be more sensitive to insect resistant plants than predators in general. It was concluded that not enough species were studied to predict the effect of a Bt toxin or proteinase inhibitor on natural enemies.

Duan, J.J., Marvier, M., Huesing, J., Dively, G., Huang, Z.Y., 2008, A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). PLos One, (No. January), p.e1415, 1932-6203.

A meta-analysis was conducted of 25 studies that independently assessed potential effects of Bt proteins on honey bee survival. The conclusion was that commercial Bt crops engineered to resist lepidopteran and coleopteran pests do not negatively affect the survival of either honey bee larvae or adults in laboratory settings.

Marvier, M., McCreedy, C., Regetz, J. & Kareiva, P., 2007, A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316, 1475–1477.
A meta-analysis of 42 field experiments (lepidopteran-resistant cotton expressing Cry1Ac protein, lepidopteran-resistant maize expressing Cry1Ab protein, and coleopteran-resistant maize expressing Cry3Bb protein) revealed that non-target invertebrates are generally more abundant in Bt cotton and Bt maize fields than in conventional fields managed with insecticides.

Nicolia A., Manzo A., Veronesi F., Rosellini D., 2013, An overview of the last 10 years of genetically engineered crop safety research. Crit Rev Biotechnol. Sep 16
Classified 1783 scientific records on GE crop safety published between 2002 and 2012.

Topic	No. of papers
General literature	166
Interaction of GE crops with the environment (G env)	847
Biodiversity	579
Gene flow (Gf)	268
Gf – Wild relatives	113
Gf – Coexistence	96
Gf – Horizontal gene transfer in soil	59
Interaction of GE crops with humans and animals (GE food&feed)	770

The Nontarget Effects of Bt Crops Database[52] is a database maintained by the National Center for Ecological Analysis and Synthesis (NCEAS). It includes publications dealing with non-target effects of GM crops modified with genes derived from *Bacillus thuringiensis* expressing one or more Cry proteins.

Review papers have covered complete risk scenarios. Two examples:

- an assessment was made on the impact of Bt maize pollen on monarch butterfly populations positioning the indication that pollen from Bt maize could harm monarch larvae (Sears *et al.* 2001)
- Tepfer *et al.* (2015) evaluate whether the expression of viral sequences in GM plants could lead to the emergence of novel viruses through recombination between transgene mRNA and that of an infecting non-target virus. They conclude that the likelihood of the emergence of novel viruses is low.

CERA protein monographs

CERA published several reviews of the environmental safety of proteins that are newly produced in GM plants. These monographs summarise and review available information extracted from publicly available sources, including peer-reviewed literature, regulatory submissions and regulatory decision documents. They are general descriptions of specific proteins that may be used in the ERA of GM crops. Information is given on the origin and function, on regulatory data specific to the protein, including any data on interactions with NTOs and observed expression levels in GM plants. Furthermore, they address the effect of the protein on the plant, including a summary of the phenotypic data describing the GM plant, data on survival and persistence, compositional analysis and gene flow. Being literature studies, these monographs may be used as a reference document in an ERA.

[52] http://delphi.nceas.ucsb.edu/btcrops/

Monographs are available for[53]:

- CP4 EPSPS protein
- Cry1Ab protein
- Cry1Ac protein
- Cry1F protein
- Cry2Ab protein
- Cry3Bb1 protein
- Cry34Ab1 and Cry35Ab1 proteins
- PAT protein
- Vip3Aa protein

Societies, conferences

The International Society for Biosafety Research (ISBR)[54] describes its aims as to promote scientifically sound research that supports biosafety assessment by improving communication among scientists who study plants, animals, and microbes with new characteristics due to altered DNA and produced using modern biotechnology.

Starting 1990, biennial meetings, the "International Symposium on Biosafety of Genetically Modified Organisms" (ISBGMO), were organised to present the results on environmental studies with GMO and to provide a platform to scientists, developers and regulators to discuss the relevance for ERA.

Similarly the West Palearctic Regional Section (WPRS) of the International Organization for Biological Control of noxious animals and plants (IOBC)[55] promotes the use of sustainable, environmental safe economically feasible, socially acceptable control methods of pests and diseases of agricultural and forestry crops. In 2003 the Working Group “GMO's in integrated plant production” was established to provide a platform for open discussions among the different parties involved, namely public sector scientists, industry scientists, regulators, decision makers and non-governmental organisations. Since the establishment of the WG, seven meetings on the "Ecological Impact of Genetically Modified Organisms" (EIGMO) have been organised and the proceedings were published.

Table 4 provides a selection of topics covered at ISBGMO and EIGMO related to the ERA of GM plants. They mark an active exchange between different stakeholders and typically resulted in scientific publications.

[53] http://cera-gmc.org/Protein_Monographs
[54] www.isbr.info
[55] http://www.iobc-wprs.org/about/index.html

Table 4 Selected topics presented at ISBGMO (2000 – 2014) and EIGMO (2003 – 2015) conferences relating to the ERA of GM plants

Organisation Conference	ISBR ISBGMO	IOBC - WPRS EIGMO
ERA methodology		
Concepts and application for ERA and regulatory decision-making	Covered	
Defining environmental harm	Covered	
Problem Formulation for ERA	Covered	
Establishment of baseline information	Covered	Covered
Predictive modelling for weediness and invasiveness	Covered	
Tiered, methodological framework for NTO ERA	Covered	Covered
Specific ERA challenges when stacking GM traits by breeding.	Covered	
Transportability of confined field trial data across national boundaries	Covered	
Meta-analysis	Covered	
Methods to Address Uncertainty in GMO ERA	Covered	
Assessing unintended effects	Covered	Covered
GM Plants – Crop/trait related		
Biosafety aspects of marker genes in GM plants	Covered	
Biosafety aspects of GM-based agronomic traits protecting against yield reduction due to biotic stress (weeds)	Covered	Covered
Biosafety aspects of GM-based agronomic traits protecting against yield reduction due to biotic stress (insect pests)	Covered	Covered
Biosafety aspects of GM-based agronomic traits protecting against yield reduction due to biotic stress (fungal diseases)	Covered	Covered
Biosafety aspects of GM-based agronomic traits protecting against yield reduction due to abiotic stress	Covered	
Biosafety consideration for crops for non-food/feed uses, biofuels and energy crops	Covered	
Biosafety aspects of introducing viral sequences in GM plants	Covered	
Biosafety aspects of RNAi mediated traits in GM plants	Covered	Covered
Biosafety aspects of GM forest trees	Covered	
GM plants – environmental impact related		
Release of transgenic crops in centres of origin or diversity	Covered	
Weediness and invasiveness	Covered	Covered
Introgression and related weediness/ invasiveness	Covered	Covered
Effect on NTOs	Covered	Covered
Microbial communities connected with GM plants	Covered	Covered
GM Plants – management related		
Strategies for biological confinement in plants	Covered	Covered
Monitoring strategies	Covered	Covered
Design and implementation of IRM programs for GM crops, including managing Bt crops	Covered	Covered

3.2 Gene therapy

3.2.1 Reports from governmental bodies

COGEM being the advisory body for the Dutch Competent Authority, publishes advices upon request relating to GM research, a.o. on specific gene therapy application dossiers[56], but also more generic advices (*e.g.* Inschaling laboratoriumwerkzaamheden met lentivirale vectoren, CGM/090331-03[57]).

Also other authorities and expert bodies have published reviews of scientific rationale to support gene therapy ERA. As an example, publications by experts of the Biosafety and Biotechnology Unit (SBB) of the Scientific Institute of Public Health in Belgium are cited:

- General Considerations on the Biosafety of Virus-derived Vectors Used in Gene Therapy and Vaccination (Baldo *et al.*, 2013);
- Environmental risk assessment of modified vaccinia virus Ankara (MVA)-based vectors used for gene therapy or vaccination (Goossens *et al.*, 2013);
- Biosafety of vectors derived from Herpes Simplex Virus Type 1 (Lim *et al.*, 2013)(SBB as co-author);
- State-of-the-art lentiviral vectors for research use: Risk assessment and biosafety recommendations (Pauwels *et al.,* 2009);
- Biosafety aspects of Modified Vaccinia Virus Ankara (MVA)-based vectors used for gene therapy or vaccination (Verheust *et al.*, 2012).

3.2.2 Research commissioned by a governmental body

The Netherlands

The formerly called Ministry of Housing, Spatial Planning and the Environment of the Netherlands, Directorate-General for Environmental Protection, Directorate for Chemicals, External Safety, Radiation Protection commissioned RIVM to assess the environmental risks of replication competent viral vectors in gene therapy trials (RIVM, 2008a). Although merely a guidance document, the report gives insight in the specificities of these vectors, their potential spread and effect scenarios. An example of an ERA is included.

Research carried out at the request of COGEM resulted in several reports (Annex 6, Table 1). The studies are diverse in nature going from inventories (CGM 2006-04 on analysis of shedding data; CGM 2010-10 on replication-competent non-human viruses; CGM 2014-08 on GM vaccines) over studies explaining methodologies (CGM 2006-04 on standardising collection of shedding data; CGM 2012-04 on ERA methodology in medical and veterinary biotechnology) to specific aspects of the ERA (CGM 2005-04 on changes in tropism in recombinant and chimeric viruses) and reflection documents on medical tourism (CGM 2010-07 on medical tourism for gene therapy in general; CGM 2011-03 on gene therapy in China). Also, reports analysing regulatory issues are included (CGM 2010-06 on potential steps towards deregulation of naked DNA applications; CGM 2012-07 specifically on ERA in clinical trials).

COGEM in cooperation with the Netherlands Society for Gene Therapy organised a workshop on vector shedding in clinical gene therapy trials (Bleijs, 2005). Vector shedding as such does not represent an environmental hazard, but a mechanism through which hazards related to the vector and/or insert may take effect. Shedding embodies the exposure element in the ERA.

The Office for Genetically Modified Organisms of RIVM ordered a study on the influence of the administration route on biodistribution and shedding of replication-deficient viral vectors (RIVM, 2008b). Not only the type of viral vector, but also the way of administration determines biodistribution and shedding. A review is presented for HAdV-5 and AAV2 and qualitative models are made to describe the processes.

[56] http://www.cogem.net/index.cfm/nl/publicaties/categorie/advies
[57] http://www.cogem.net/index.cfm/nl/publicaties/publicatie/inschaling-van-laboratoriumwerkzaamheden-met-lentivirale-vectoren

Another study for RIVM addresses effects of immune modulation in gene therapy (Aerts Kaya, 2010). Immune responses play a role in clearance of viral vectors. Some vector types induce a rapid response. To reduce the negative effect on clinical efficacy of these vectors, immune modulatory agents are used. However, their administration may also increase the risk for infections, recombination with wild type virus and affect biodistribution, persistence, shedding and transmission. Longer persistence of the vector may increase the duration of interaction with wild type viruses and recombination and potentially increase the risk and/or duration of shedding of administered viral vectors.

Other EU Member States

Also in other EU Member States governmental bodies commissioned some research in support of gene therapy ERA. The following are two examples from the UK.

A study for DEFRA performed by Atkins Environment (2008) discusses generic hazards posed by the use of live GMOs in medicines (viruses and bacteria):

- Pathogenicity of the GMO - the ability of the GMO to cause disease, either within the recipient, or to the wider environment following any release of the GMO;
- Production of biologically active and/or toxic products by the GMO, or by the recipient in response to the presence of the GMO;
- Non-target effects by the GMO, or as a consequence of the presence of the GMO;
- Genetic instability of the modifications, both deletions and additions;
- Changes in cell, tissue and host tropism of the GMO;
- Gene transfer (horizontal and vertical) of the added genes (the transgenes); and
- Survival and dissemination of the GMO in the environment.

The report also lists gene therapy applications in clinical trials (up to July 2007).

In response to an initiative from DEFRA for a desk study to review environmental risks from research trials and marketing of GM veterinary and human medicines, the UK Food and Environment Research Agency (FERA) report (2012) inventoried emerging technologies in GM human and veterinary medicine development and the potential emerging risks associated to these techniques. Risk factors, adverse effects and likelihood of a diversity of vectors and targeted diseases were addressed in general terms. At the project's workshops conceptual model diagrams were drawn that show the causal chain with its influencing factors leading to an adverse effect. However, it was stressed that the actual risk assessment should be done on a case-by-case basis. In the light of the possibility of gene transfer in the gut, it was stated that research is needed to increase knowledge of gut flora in humans and animals in order to evaluate the impact of such an event. In general it was concluded:

> "*The lack of published references specifically relating to environmental risks of GM medicines was thought to be due to the fact that the technology is relatively new, and very few GM medicines have been released for marketing to date*."

3.2.3 Government funded research projects

In the EU funded Framework Programmes several projects relating to health and dealing with gene therapy were funded. A selection of the projects is presented in Annex 6, Table 2. Included are projects that deal with safety although it is not clear from the information provided on the Cordis website[58] whether environmental safety is incorporated. Furthermore, projects that include clinical trials are listed as they have to address environmental safety in the trial applications.

Apart from plasmid DNA, a diversity of virus vectors is studied for safety and efficacy. Virus systems include *Adeno-Associated Virus*, adenoviruses, alphavirus (*e.g. Semliki Forest virus*), gamma-retrovirus, herpes virus, lentivirus, *Porcine Coronavirus, Porcine Parvovirus,* pox virus, spumavirus and *Vaccinia virus*. The research involves basic as well as more advanced research

[58] http://cordis.europa.eu/projects/home_en.html

up to clinical trials. One project, ClineGene[59], was established to facilitate and help harmonise ethical, quality, safety, efficacy and regulatory issues by establishing standards for clinical gene transfer products. Especially quality and safety are tackled. The project's website also holds 3 databases for gene therapy.

While safety may be focussed on the patient, several elements also serve the ERA. None of the listed projects has environmental safety as its main subject.

3.2.4 Information from applications

Clinical trials

August 2015 the JRC database on deliberate release of GMOs other than plants[60] lists 165 gene therapy clinical trial applications submitted since 17 October 2002. Table 1 in Annex 7 gives an overview of the type of vectors and inserts that are used. This list contains only a part of the gene therapy trials in the EU as only clinical trials classified as deliberate release are published on the JRC website. Each trial record is provided with a SNIF document that gives a summary of the ERA conducted by the applicant. Only in a few cases a final report of the trial is available.

The Journal of Gene Medicine published by Wiley[61] maintains a worldwide database on gene therapy clinical trials. The update of July 2015 includes a cumulative total of 2210 gene therapy trials.

In Table 5 the results from an analysis of the JRC reported "deliberate release" clinical trials in the EU and from the worldwide database are compared. Most EU trials aim to develop and test a treatment for cancer, followed by treatments for or vaccines preventing infectious diseases. Hereditary diseases come at the third place. The most common vectors are based on *Vaccinia virus* and adeno-associated viruses, followed by adenoviruses. Also plasmid DNA and bacteria are used as vector. The global cumulative database confirms that cancer is the primary target for gene therapy trials. Next are investigations for monogenic diseases and an equal amount of trials for cardiovascular diseases followed closely by infectious diseases. The distribution of vector types is different from what is observed in the EU trials. Adeno- and retroviruses come first with naked/plasmid DNA on the third place.

Table 5 Number of clinical trials sorted per type of disease addressed, type of vector used based on the EU GM database (search performed in August 2015) and the worldwide database (Journal of Gene Medicine update July 2015)

	Number of clinical trials	
	EU	**Global**
Type of disease addressed		
Cancer	77	1155
Cardiovascular disease	16	150
Infectious disease	45	142
Inflammatory disease	3	13
Monogenic disease	21	151
Neurological disease	2	36
Other (*e.g.* ocular diseases, gene marking, healthy volunteers)	1	139
Type of vector used		
Viruses	137	1516
Adeno-associated virus	31	137

[59] http://www.clinigene.eu/
[60] http://gmoinfo.jrc.ec.europa.eu/gmo_browse.aspx
[61] http://www.abedia.com/wiley/

	Number of clinical trials	
	EU	Global
Adenovirus	25	506
Herpes simplex virus	14	73
Lentivirus	8	114
Retrovirus	5	420
Vaccinia virus	42	165
Other (*e.g.* poxvirus)	12	101
Plasmid DNA	15	397
Bacteria	13	-
Other (*e.g.* lipofection, unknown)	-	365
Type of genes used		
Antigen	63	446
Cytokine	13	358
Receptor	8	208
Deficiency	33	191
Tumour suppressor	19	171
Suicide	2	163
Growth factor	34	163
Replication inhibitor	-	92
Marker	8	55
Other categories	9	308
Unknown	-	55

For the clinical trial applications in the Netherlands the detailed ERA can be found in the GGO Vergunningendatabase[62]

Commercial approvals

The first gene therapy product approved for clinical use in humans is Gendicine in 2003. It is manufactured by Shenzhen SiBiono GeneTech in China. This recombinant adenovirus is modified to express wild-type-p53 tumour suppressor gene (Ad-p53) for treatment of tumours with a mutated p53 gene (Peng, 2005). An extensive clinical gene therapy program using rAd-p53 in the treatment of head and neck squamous cell carcinoma predated the approval starting in 1998. The product was proven to be safe. The most commonly observed side effects were self-limited fever in approximately one third of Gendicine treated patients.

Oncorine gained regulatory approval in China for nasopharyngeal carcinoma combined with chemotherapy in 2005. This GM adenovirus produced by Shanghai Sunway Biotech is a replication-competent adenovirus defective of the E1B-55kDa gene thereby relying on the cytotoxicity that is associated with the viral propagation within cells (Ma *et al.*, 2008). Again, fever, local pain at the injection site, and flu-like symptoms were frequently reported, but were not considered a serious adverse effect.

In the EU Glybera made by uniQure was approved in 2012. Treatment with Glybera aims to compensate for lipoprotein lipase deficiency. The accompanying ERA was taken as an example in Chapter 2.1.

Imlygic (Talimogene laherparepvec, T-VEC) developed by BioVex, Inc. (now Amgen) was recently approved by FDA in the USA. T-VEC is a modified *Herpes Simplex Virus 1* for the treatment of melanoma in patients with inoperable tumours. This oncolytic virus secretes the cytokine hGM-CSF selectively in tumour cells.

[62] http://www.ggo-vergunningverlening-zoeken.nl/

3.2.5 Scientific reports/publications

While publications on clinical trials are plentiful, review articles on safety for the environment and humans other than the patient are scarce. However, studies on patient safety are also informative for the safety assessment of medical staff and family members. The Thematic Issue: Biosafety of Viral Vectors Commonly Used in Gene Therapy and Vaccination' of the Journal Current Gene Therapy in 2013[63] provides some excellent overviews.

Box 9 Examples of publications relating to biosafety of gene therapy

Van den Akker E., van der Vlugt C.J., Bleijs D.A., Bergmans H.E., 2013, Environmental risk assessment of replication competent viral vectors in gene therapy trials: potential effects of inserted sequences. Curr Gene Ther. 13(6): 395-412.
The article describes a methodology for the ERA of replication competent viral vectors. Due to the higher potential for exposure the characteristics and context of the insert, especially inserts produced by synthetic biology, are taken into account.

Myhr A.I. and Traavik T., 2012, Genetically Engineered Virus-Vectored Vaccines – Environmental Risk Assessment and Management Challenges. In "Genetic Engineering - Basics, New Applications and Responsibilities" H.A. Barrera-Saldaña (Ed.), p.199-224.
The book chapter gives an overview of the type of vaccines and applications currently used. It furthermore points to the difficulties in performing an ERA for viruses as they are neither chemicals nor real organisms. Examples are given of potential hazards and related studies. The authors denounce that so far no GM virus vaccines have been thoroughly risk assessed from an environmental point of view.

Schenk-Braat E.A., van Mierlo M.M., Wagemaker G., Bangma C.H., Kaptein L.C., 2007, An inventory of shedding data from clinical gene therapy trials. J Gene Med. 9(10): 910-21.
The study provides a broad inventory of published shedding data from historical clinical trials to support evidence-based risk assessment. Due to non-standard, non-quantitative tests, the lack of information on assay sensitivity, no general conclusions can be drawn.

Kawahira H., Matsushita K., Shiratori T., Shimizu T., Nabeya Y., Hayashi H., Ochiai T., Matsubara H., Shimada H., 2010, Viral shedding after p53 adenoviral gene therapy in 10 cases of esophageal cancer. Cancer Sci 101(1): 289–291.
A study where 10 oesophageal cancer patients were examined for shedding.

Brandon E.F., Hermsen H.P., van Eijkeren J.C., Tiesjema B., 2010, Effect of administration route on the biodistribution and shedding of replication-deficient AAV2: a qualitative modelling approach. Curr Gene Ther. 10(2): 91-106.
This review presents a critical overview on biodistribution and shedding data. Based on these data, a qualitative model for the biodistribution and shedding of AAV2 based viral vectors is presented. This can help in deciding which studies are warranted or which safety precautions are needed after administration to patients.

Tiesjema B., Hermsen H.P., van Eijkeren J.C., Brandon E.F., 2010, Effect of administration route on the biodistribution and shedding of replication-deficient HAdV-5: a qualitative modelling approach. Curr Gene Ther. 10(2):107-127.
The same exercise if applied to replication-deficient HAdV-5 as above.

[63] http://benthamscience.com/journal/contents.php?journalID=cgt&issueID=119403

Societies, conferences

In contrast to organisations addressing the ERA for GM plants, safety aspects of gene therapy have at most been handled as a side-element of other initiatives: *e.g.* Gonin *et al.* (2005) report on gene therapy biosafety discussed during the round table of the 2nd European Conference & Practical Course: Towards Clinical Gene Therapy: Preclinical Gene Transfer Assessment. While they address in detail patient safety and the regulatory framework including the EU GMO requirements, there is no mention of ERA aspects.

Similarly, while societies like ISBR covered ERA aspects of GM microorganisms, these were almost exclusively related to GMOs to be intentionally released (*e.g.* for bioremediation) or accidently discharged (*e.g.* from production facilities).

3.3 Conclusion

The information presented in this section is a sample of the different types of data that have been accumulated for GM plants and gene therapy applications, and that can contribute to an informed ERA. Different stakeholders have participated in gathering data, critically reviewing results and debating their usefulness for risk assessors. Whereas the introduction of GM legislation was marked by uncertainty over the possible impact of GMOs, the ERA conducted over thirty years of field trials with and twenty years of commercial cultivation of GM crops, and the fact that adverse effects have never been observed after two decades of GM cultivation, as well as ERA related to thousands of gene therapy trials should enable a more focussed approach to areas of concern.

This overview indicates that there is far more information relevant for ERA of GMHP plants than for the ERA on gene therapy. Possible factors influencing this bias include:

- Prominent environmental exposure of GM plants as opposed to gene therapy
  With first GM plant applications targeting major commodity crops, introduction in the environment was fundamental. It was already integrated in the R&D phase. Hence, ERA had to be conducted and provided important indications along the step-by-step approach. On the contrary, many gene therapy trials were conducted in a contained use environment with specific measures to avoid exposure and release. Even clinical trials performed under Directive 2001/18 are in practice carried out in the contained environment of a hospital and/or with additional risk management measures to prevent release. ERA related questions and associated studies could thereby be postponed. While this enabled research projects to carry on, it also provided fewer insights in the behaviour and potential environmental impact of the gene therapy product. Furthermore, the scope of the potential impact was narrowed to the patient, clinical trial staff and related people, rather than broadening to the environment.

- Legal regime
  In many countries, the legal approach for gene therapy trials is based on contained use legislation (or the local equivalent). This approach further strengthens the previous point as the process largely looks at containment efficiency rather than to the question if the containment is required as a result of an ERA. Also, there may be different authorities and expert advisory bodies dealing with contained uses and deliberate releases, marking the difference between the two situations. On the other hand, field trials with GM plants are globally considered as introductions in the environment. Although the scale of a trial is taken into account, an ERA will be conducted in each case.

- More advanced GM crop projects and products
  It can be argued that both GM plants and gene therapy open new options for their respective targeted challenges. However, more detailed analysis reveals that GM plants offer a new approach to increase germplasm variability: once the novel trait has been introduced, the conventional processes of plant breeding are fully applicable and are necessary to ensure that varieties adapted to local conditions can be offered. Gene therapy, on the contrary, offers a completely new paradigm for delivery as well as

identification of the therapeutic product. It can therefore easily be understood that GM plants could relatively quickly be integrated in the seed markets.

In consequence a high number of field trials and products on the market mark the adoption of GM crops. Gene therapy has up to now only 4 commercial authorisations (Glybera in the EU, Imlygic (T-VEC) in the USA and Gendicine and Oncorine in China). Although worldwide the number of clinical trials is high - 2210 in July 2015 (Wiley[64]) – this does not compare with the experience built up with GM plants. As a result fewer data are available that may serve the ERA for clinical trials.

- Global scale of GM crop products
  Major commodity crops are widely deployed and hence require conventionally testing in different climatic and environmental situations. Limiting factors (*e.g.* weather conditions) and interacting environmental elements (*e.g.* sexually compatible species, NTOs) may differ depending on the location where a GMO is introduced. Questions have been raised if releases in centres of origin present unique challenges. The interaction of a GM crop with its environment therefore seems clearly dependent on the specifics of the environment and may require testing under a diversity of situations. Recently discussions on data transferability have started, providing an indication under which conditions information collected in one country will be relevant for other countries/environments.
  In many cases, the environment for gene therapy products was perceived as limited to the clinical setting and the immediate surrounding, with humans as primary target as well as NTO. Such situations may seem less diverse, therefore requiring less diversity and specification in the ERA supporting information.

- Interest of the scientific community
  GMOs not only triggered basic research questions, they also provided new tools and impetus for that research. The use of genetic markers, some that could easily be screened, allowed experimental designs which beforehand had been difficult to perform. *E.g.* pollen flow studies and interspecific crosses were enabled by markers such as herbicide tolerance that allowed retrieving low frequency events. At the same time, the environmental safety of GMOs justified ecological studies that beforehand had received only limited interest. In spite of a long history of safe use of crop plants, little was known on how crops behave in unmanaged environments and this prompted more questions on what factors can make plants invasive or behave as weeds. While such data can support the ERA, the researchers may have been motivated more by their scientific interest rather than an environmental concern. It can be questioned if such detailed investigations were fully warranted by the precautionary approach and if GMHPs presented sufficient risk to reasonably require such a fundamental approach.

  For gene therapy the interest in baseline information is less straightforward. Working with vector systems derived from pathogens, the focus is on eliminating negative features and making efficient and safe vectors, rather than studying their environmental behaviour.
  Information on these “crippled” derivatives may be of limited value for understanding the behaviour of wild-type organisms. In many cases the route of transmission/administration and subsequent fate will differ fundamentally. As such research on gene therapy products is more directly motivated by environmental concerns, which in all cases is deemed to be focussed on safety of humans (target and non-target persons).

  Finally, the focus on safety research also provided research funds in disciplines and at a time when funding was becoming scarce. Whereas a researcher will be interested in furthering knowledge, a risk assessor will be satisfied with a level of information sufficient for the ERA. *E.g.* a researcher may want to document the exact frequency of an introgression event, whereas a risk assessor may suffice with knowing that a specific interspecific cross occurs.

[64] http://www.abedia.com/wiley/

- Heightened critical public attention for GM plant applications
  Deployment of GMOs in the environment attracts more public attention than contained activities. In addition, it has been argued that people are willing to accept more (perceived) risks in relation to healthcare products than for changes in agronomic practices. This is exacerbated by the fact that healthcare is already perceived as highly technical and controlled whereas the agri-food sector promotes traditional and environmental-friendly approaches. The debate over GM crops has crystallised over the years, resulting in a stalemate between stakeholders, each using scientific findings to illustrate their positions.

  E.g. in 1995 the US EPA considered data on Bt maize's impact on NTOs (U.S. Environmental Protection Agency Publ. No. EPA731-F-95-004) found no threat. In June 1999, Losey *et al.* reported a preliminary study as a note in Nature, indicating that monarch butterflies under laboratory conditions might be harmed by eating pollen from Bt maize plants. The finding led to detailed research on different aspects of the risk scenario including Bt expression levels in pollen, maize pollen deposition on milkweed in and near maize fields, temporal and spatial overlap between monarch larvae appearance and maize pollen shed, monarch larvae sensitivity to purified Bt proteins and pollen and the impact of Cry1Ab-expressing maize pollen on monarch butterfly larvae in field studies (first publications in Proceedings of the National Academy of Sciences 2001, vol. 98 no. 21). None of these studies has changed the original ERA, which predicted negligible impacts due to the low exposure of non-target Lepidoptera to pollen or other plant tissue containing Cry1Ab under agricultural conditions (CERA, 2011c).

As information on GM crops accumulated, monographs, reviews and meta-analyses became available. Recently, systematic reviews have been introduced as a method to synthesise information in function of a specific goal such as the ERA. For gene therapy products the information is limited and more specific for particular cases. Few reviews are available.

4 Experience leading to confidence

In this section an attempt is made to identify elements for which an ERA can be conducted with acceptable confidence and no additional information is needed. The authors acknowledge that the final decision on what is required for an ERA is ultimately and solely determined by the risk assessors when performing a specific ERA. This analysis therefore focuses on elements for which additional research is not likely to provide new insights and/or to improve the quality of the ERA. As always with general conclusions, exceptional cases may nevertheless require revisiting these suggestions.

4.1 Uncertainty versus confidence

While research continuously advances scientific knowledge, there will always remain boundaries to what can be deduced with a level of confidence. Addressing scientific uncertainties on risks is the core element of the precautionary principle:

> *In situations where risks are uncertain, the precautionary principle assumes that risks could be present, and efforts should be made to protect human health and the environment* (CPB, 2012).

Uncertainty may result from the inability to predict the possible hazards or may be a consequence of the lack of sufficient knowledge to estimate with confidence the possible impact associated with the activity. In any case it must be assumed that the protection goals are clear from the start, which as pointed out before, may not yet be fully the case. While protection goals can be redefined over time, a lack of clarity on what they are and what effects would be deemed acceptable makes an impact assessment meaningless. Even if an effect would be observed, it would be impossible to determine if the effect is relevant and undesired.

Uncertainties may arise from:
(i) lack of information,
(ii) incomplete knowledge, and
(iii) biological or experimental variability, for example, due to inherent heterogeneity in the population being studied or to variations in the analytical assays.

Uncertainty resulting from lack of information includes, for example, information that is missing and data that is imprecise or inaccurate (*e.g.*, due to study designs, model systems and analytical methods used to generate, evaluate and analyse the information) (SCBD, 2012).

In developing an ERA there may be aspects that cannot be studied or where study results do not give clear information (yet). Commission Decision 2002/623/EC explains uncertainty and how to include it in the ERA as follows:

> *'ERA has to take into account uncertainty at various levels. Scientific uncertainty results usually from five characteristics of the scientific method: the variable chosen, the measurements made, the samples taken, the models used and the causal relationships employed. Scientific uncertainty may also arise from a controversy on existing data or lack of some relevant data. Uncertainty may relate to qualitative or quantitative elements of the analysis. The level of knowledge or data for a baseline is reflected by the level of uncertainty, which has to be provided by the notifier (assessment of uncertainty, including lack of data, knowledge gaps, standard deviation, complexity, etc.) in comparison with the scientific uncertainties in current practice.'*

Mid 2015, the EFSA Scientific Committee launched a public consultation[65] on "Guidance on Uncertainty in EFSA Scientific Assessment". The document illustrates how EFSA intends to include considerations of uncertainties in its scientific assessments. The Guidance will be applicable to all areas of EFSA and all types of scientific assessment. It does not prescribe

[65] http://www.efsa.europa.eu/en/consultations/call/150618

specific methods for uncertainty analysis but rather provides a harmonised and flexible framework within which different methods may be selected, according to the needs of each assessment.

When an uncertainty is identified that has a significant impact on the outcome of the ERA, different responses can be envisaged:

- More research can be performed to provide additional information and reduce the level of uncertainty,
- Management measures can be put in place to reduce the chance that the risk materialises, and/or
- Monitoring activities can be conducted.

One of the challenges for risk assessors is to determine which uncertainties have a significant impact on the outcome of the ERA. Conversely, it must be questioned if more information will significantly contribute to the quality of the ERA. Johnson *et al.* (2007) commented that

> *"collecting data and making vague assertions that they are relevant to risk assessment, without providing specific predictions about things of concern, only serves to confuse and increase unease."*

Craig *et al.* (2008) recognised the potential for environmental risk assessment research to overwhelm regulators, and thereby increase the time taken to evaluate regulatory dossiers:

> *"In the decade since the first authorizations for commercial release of transgenic crops, there has been an enormous increase in the amount of data generated by scientific studies that relates to risk assessment. If this trend continues, we run the risk of competent authorities being submerged by excessively large amounts of data that may be of questionable pertinence to verifiable safety questions."*

As pointed out before, more information may not automatically lead to better ERA. At the same time, while new or more detailed information may render an ERA more robust, only in specific cases it will lead to an adjustment of the ERA. The challenge for determining information requirements therefore concentrates on the balance between the effort to acquire more information and the (expected) pertinence of that information for the ERA. Also, if the worst-case scenario indicates an acceptable risk, then further studies for obtaining quantitative data may be unnecessary (Anliker *et al*., 2009).

We structure the topics based on a schematic presentation of risk scenarios (Figure 2), including 3 major parts:

- Defining the stressor/activity
- Mechanisms that can lead and/or contribute to an undesired effect
- Evaluating the impact on an environmental protection goal

The areas of concern are included where appropriate.

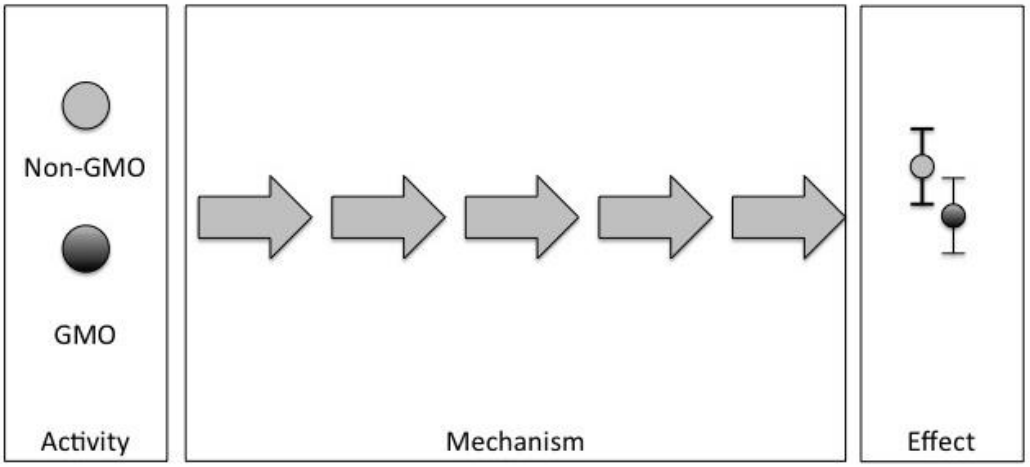

Figure 2 Schematic representation of "risk scenarios"

4.2 Stressor/activity

The comparative assessment acknowledges that the ERA of most activities with GMOs can build on what is already known for the non-modified host/parental organism. It recognises that the engineered modifications only change specific aspects of the organism. When the host organism has a history of safe use (or rather "a use for which the impact is accepted"), the ERA can focus on the potential impact resulting from the modification. While this approach has been successfully applied, the question remains on what approach should be followed for products that are not eligible to rely on the comparative approach.

4.2.1 GMHP

Although genetic modification broadens the scope of genetic changes that can be introduced into plants, it does not inherently result in plants that are less safe than those produced by more conventional techniques (National Academy of Sciences, 1987 and 1989). Their degree of riskiness or safety depends on the characteristics of the inserted gene(s), the final organism that is produced and the application to which it is put. There is no scientific evidence that the use of the technology is itself inherently unsafe.

The extensive number of datasets that has been generated in the past 30 years in relation to GMHPs confirms that the genetic modification *per se* is not introducing a specific or unique hazard. This confirmation has led to questioning if subjecting GMO as a legally defined class to a specific ERA is justified or if other approaches should be explored. In 2015 USDA has initiated a process to modify its regulatory approach for GMOs. One of the aspects of the change will be the definition of a new "regulatory trigger" (*i.e.* the conditions that determine if an activity or a product is within the scope of application). This new trigger will be better adapted to capture the very small amount of applications that might pose a risk, rather than covering a broad range of products that don't pose a risk but legally require an ERA. Also in the EU the discussion on the interpretation of the legal definition of GMOs has inspired a broader reflection of the basis of the legislation.

Another aspect, which is prominent in working with plants, is the introduction of stacked products obtained via natural crosses. As pointed out before, different authorities have adopted divergent approaches. In the EU each stack has to be approved and higher number combination stacks can only be approved when individual events have been successfully evaluated.

Based on the information obtained so far, we conclude that most stacks will not create new issues requiring necessarily a new ERA and propose a more targeted approach with an incremental ERA limited to cases where the genes/ traits introduced by the individual events are expected to interact with each other, possibly leading to a new of a synergistic effect:

- For stacked events combining *e.g.* herbicide tolerance and insect resistance no interaction of the pathways, mode of actions and/or effects is anticipated. No additional ERA is required.
- When combining multiple insect resistance genes a more prudent approach may be warranted (CGM 2014-02, CGM 2014-05). Several stacks combining 2 or more genes conferring insect resistance have been approved.

Crops

A diversity of documents (biology documents, guidance documents, publications ...) illustrate that this topic is well covered. Driven by environmental considerations for GM crops, additional information has been gathered on non-agronomic aspects of crops which beforehand had been less investigated. Biology documents are prepared building a baseline for comparison of GM events. Market application dossiers including industry funded safety studies are submitted in several countries and numerous research papers are published including from publicly funded projects. Methodology has been published on how to perform the comparative analysis. The fact that the non-modified crops with a history of safe use can serve as reference in the comparison has been fundamental in this approach.

The best documented plant species are the big row crops, like maize, cotton, soybean and oilseed rape. It is hard to conceive which additional information would be required in order to substantially improve an ERA for any of these species. Furthermore, they provide a model for information requirements for other plant species.

Traits

Herbicide tolerance

Herbicide tolerance is the main trait in commercialised events and is well documented. Each of the areas of concern has been addressed for the most frequent herbicide tolerance mode of actions, *i.e.* glyphosate tolerance conferred by the bacterial *CP4 epsps* gene and glufosinate ammonium tolerance by the *pat* gene (CERA, 2011a, 2011d). ERAs are performed for at least 30 *CP4 epsps* events and stacks, and 38 for *pat* events and stacks. Market authorisations have been obtained around the world for 94 events for *CP4 epsps* and 99 *pat* transformation events, single and stacked[66]. This leads to the following conclusions:

- The modes of action provide tolerance to agronomic relevant dosages of respective herbicides. Importantly, there is no cross-protection beyond the class of plant protection products: *e.g.* with *CP4 espsps* the GM plant is protected against a glyphosate treatment while remaining susceptible to glufosinate treatments. Conversely glufosinate tolerant plants remain susceptible to a glyphosate treatment. The tolerance is highly specific.
- The selective advantage is limited to areas treated with the specific herbicide to which tolerance is conveyed (*i.e.* managed and in some cases semi-managed areas). In agricultural environments volunteers may cause an agronomic issue, if not adequately managed. Although pollen flow does occur (projects in FP1-BAP and FP2-BRIDGE, CGM 2003-02, CGM 2005-05, CGM 2007-06), the hybrid plant population rapidly loses the trait as no selection pressure is present in a non-agricultural environment.
- The trait has no side effect on other plant characteristics: the gene products themselves do not influence characteristics such as seed dormancy, shattering, competitiveness and gene flow. Adding this trait to domesticated crops therefore does not make crops more persistent or invasive.
- Aspects of safety of plant protection products are handled via specific legislation, in particular Regulation (EC) No 1107/2009 on plant protection products. EFSA (2008) clarified how the interaction between the plant protection product legislation and the GMO legislation is organised.
- Impacts on human and animal health through contact or incidental intake are not demonstrated. CP4 EPSPS and PAT are rapidly degraded in mammalian digestive systems or in experiments simulating gastric environment, and have no significant sequence or structural homology to known toxins or allergens. Phenotypic characterisation, compositional analyses and nutritional analyses show that the proteins have no characteristics that might impact other organisms.

Insect resistance

The second important trait is insect resistance, of which the most studied are products based on *cry1Ab*, *cry1Ac* and *cry34Ab1*/*cry35Ab1* genes (CERA, 2011b, 2011c, 2013) (Table 6).

- Phenotypic data do not show any change in characteristics of the plants that might contribute to its survival or persistence.
- The potential for weediness in agricultural and non-agricultural environments is not affected by the introduction of the Cry proteins. Although Cry proteins provide protection against certain pests, this trait alone is not considered important enough for plants to become invasive.
- The gene flow is not affected by the genetic modification and neither the potential for gene flow to impact the weediness of wild relatives.

[66] http://www.isaaa.org/gmapprovaldatabase/default.asp, last accessed November 3, 2015.

Table 6 Number of events and stacks with the most common insect resistance genes

Events and stacks incorporating most commonly used *cry* genes for which the environmental risks have been assessed and which have been approved in at least one country[67].

Genes	Protection against	# Events and stacks for which an ERA is available	# Events and stacks approved in at least one country
cry1Ab	Lepidoptera	17	62
cry1Ac	Lepidoptera	14	36
cry34Ab1/*cry35Ab1*	Coleoptera	2	35

While maintaining a case-by-case approach for ERA, as required in many countries, a more simplified regulatory process may be considered for well-known crops and genes. *E.g.* dossiers for new events that combine a new trait with an herbicide tolerance induced by an already assessed and approved gene may require less information relating to the herbicide tolerance. Also new crops modified with well-documented genes should benefit from the available data.

4.2.2 Gene therapy

Gene therapy applications

The approach for gene therapy is fundamentally different from GMHP. Only in specific gene therapy cases the parental organism has a history of safe use, *e.g.* vectors derived from MVA, that has a history of save uses as a vaccine. Rather, in most applications, the non-modified recipient organism is related to a pathogen, which can be strongly attenuated and/or to which the population may have built up immunity. Starting from the native form, a number of modifications were required to remove or reduce essential pathogenic characteristics while maintaining those features that allow the system to function as a vector. The comparative approach must therefore identify the new intended modification, while at the same time confirm that pathogenic features have not been restored or that new ones have not been inadvertently introduced. This makes the assessment of the modifications more complex compared to GMHP.

The development of gene therapy further contrasts with the advance in GM crops as gene therapy R&D is from the start marked by a greater diversity of traits and genetic strategies. The possibility to compare and integrate information is furthermore hindered by the fact that in contrast to information obtained in publically funded research projects, information presented by applicants for their product may be subject to confidentiality. With only one gene therapy product on the market in Europe and one in the US, less experience concerning environmental risks is gained compared to GM crops.

The many clinical trials, however, have resulted in considerable information. As indicated in the previous chapter, some initiatives analysed the available information in relation to ERA.

Gene therapy vectors

Many types of vectors are used and reviews for some frequently used vectors are available: MVA vectors (Goossens *et al*., 2013; Verheust *et al*., 2012), lentivirus derived vectors (Pauwels *et al*., 2009; Rothe *et al*., 2013), human adenovirus (Wold & Toth, 2013), adeno-associated virus (AAV) (Dismuke *et al*., 2013) and *Herpes simplex virus* vectors (Lim *et al.*, 2013), These reviews are to a certain extent comparable with the biology documents made for crops.

In contrast to crops that are not pathogenic, virus vector parent organisms are classified as risk class 1 to 3, the derived vectors as 1 or 2 (Baldo *et al*., 2013).

- Virus vectors that belong to risk class 1 may be eligible to a simplified ERA procedure. An example is the MVA that is highly attenuated. It does not integrate into the genome and is

[67] http://www.isaaa.org/gmapprovaldatabase/default.asp, last accessed November 3, 2015.

localised in the cytoplasm. It propagates with difficulty in mammalian cells. The possibility for the vector reverting to wild-type is commonly accepted as negligible.

- In the Netherlands naked DNA applications benefit from a simplified procedure.
- Current lentiviral vectors (*e.g.* 3rd generation self-inactivating (SIN) systems) are an example for risk class 2 vectors. The main issues are the potential generation and propagation of replication competent lentiviruses during vector production and the capacity to integrate into the genome potentially inducing mutagenesis.

The nature and stability of attenuating modifications are an important factor to consider in the ERA, as they may significantly reduce or alter the pathogenicity of the gene therapy vector. Usually, the origin and nature of attenuating modifications will be well understood and will form an important part of the risk assessment. In some instances, however, the nature of the attenuation may not be well understood but the wild-type or parent GMO may have a history of safe use. For example, many vaccinia virus strains have been modified by passage, and have not been fully characterised, but have been used extensively in human vaccines.

Replication competent viruses retain characteristics that make them able to multiply within a treated person and therefore a basic principle in the risk assessment of these viruses should be that there is a chance of spreading into the environment. Gene therapy using *ex-vivo* genetically modified cells and/or replication-incompetent viruses are considered to pose no or limited environmental risks. Characterisation of any potential replication-competent recombinants that might be generated during manufacturing is therefore important from both a product quality standpoint and for consideration of the effect of shedding.

These considerations and providing that all applications would use the same vector background, at first sight, would allow performing the ERA as a group according to the type of vector. This can provide an option for streamlining applications and regulatory procedures while maintaining a high level of protection. It must however be observed that while vectors are improved to have enhanced efficacy and safety profile, this diversity makes generalisation difficult.

Gene therapy traits

The nature of the insert may change the assessment of the virus vector. Genes encoding cytokines, toxins or virulence factors present challenges for the ERA. Moreover, compared to GM crops a multitude of inserts has been used. As a result, a case-by-case approach for the evaluation of the traits is still needed. With more information on gene therapy ERA becoming available, it will be possible to discern types of traits that share common features and can be evaluated in a similar way.

4.3 Mechanisms

“Mechanisms” are causal chains of events through which (indirect) effects on human health or the environment can occur. While the comparison between a GMO and its counterpart may reveal a difference that potentially can lead to an undesired outcome, the probability of such an event will be determined by the likelihood of each step of the causal chain. Several causal chains may have common steps. Illustrating that it is unlikely for specific steps to occur would suffice to exclude a possible desired effect.

Whereas mechanisms may be presented in quantitative terms, the ERA may be focussed on qualitative approaches. *E.g.* when a pollen flow study shows that there is a frequency of 1/100.000 to detect a successful hybridisation at 200 meters from the pollen source, the ERA will probably take the outcome of the scenario, the so-called “worst case” scenario, into account even if it has a small probability.

This structure of this aspect is based on the guidance notes on the objective, elements, general principles and methodology of the ERA referred to in Directive 2001/18/EC[68].

4.3.1 GMHP

- The spread of the GMO(s) in the environment
  The presence of a GM plant in a certain environment can be a consequence of an intended introduction (sowing of seeds/ propagating material in fields), the consequent movement of pollen and distribution of seed or propagules to other fields and the wider environment, or the consequence of a release of viable propagating material during import, transportation, storage, handling and processing. Crop biology documents and an array of studies cover geographical dispersal via pollen as well as geographical and temporal dispersal via seeds or survival structures. These mechanisms are well understood and only in specific cases, *e.g.* when the GM trait influences relevant pollen characteristics or seed survival, additional information may be needed.

- The transfer of the inserted genetic material to other organisms
  For most crops, the hybridisation and introgression potential with sympatric compatible relatives is sufficiently documented. More information would only be warranted if the modification would intentionally target to change the out-crossing and introgression capacity.

  Horizontal gene transfer (*i.e.* gene transfer not transmitted to off-spring) for GMPH is highly unlikely. Further investigation is only justified for cases when the transfer potential would be intentionally enhanced or when the effect of a transfer would be deemed unacceptable. This can be determined on the basis of the acquired characteristic and the prevalence of similar traits in microbial communities.

  Another aspect closely related to gene transfer is the question if recombination could lead to new combinations. *E.g*. the concern was raised that insertion of viral sequences in the plant genome would lead to the emergence of novel viruses through recombination between transgene mRNA and that of an infecting non-target virus. The risk scenario that was developed by Tepfer *et al.* (2015) can serve as guidance for similar concerns.

- Phenotypic and genetic instability
  Stability of the newly introduced traits is part of product characterisation. Developers have a clear interest in selecting only those GMHP that have proven to be stable. Furthermore, during the deployment phase, quality control mechanisms are in place to confirm stability. There is no indication that traits introduced via genetic engineering would be more or less stable than conventional traits. The ERA can therefore assume a natural level of instability and may focus on those situations were instability would lead to a safety concern.

- Interactions with other organisms
  Some GM characteristics in GMHPs specifically target other organisms (*e.g*. insect resistance). Substantial documentation has been accumulated on the possible reaction of the target organisms as well as on the management of the development/selection of resistance in the insect population. It must be specified that most of these studies relate to the application of Bt proteins, and even more specifically Cry1Ab delta-endotoxin. This protein is present in GM cotton, maize and rice. Additional scientific research as well as experience from large scale release continues to broaden the range of NTOs exposed to these proteins and increases the level of confidence, but unless an unexpected counter-indication would be identified, there is no justification to expand the requirements for pre-authorisation NTO testing.

[68] Commission Decision of 24 July 2002 establishing guidance notes supplementing AnnexII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ 30/07/2002 L200/22)

- Changes in management
  The introduction of GM plants for cultivation may require specific management practices and cultivation techniques and, may lead to additional changes in management and production systems. These in turn can have an effect on protection goals. The introduction of a herbicide tolerance or an insect resistance extends the options for respectively weed and pest management. Herbicide tolerant crops support no or reduced tillage of the soil, whereas the adoption of insect resistant GM crops in the USA resulted in a decrease of insecticide uses (CGM 2012-09). Studies so far confirmed the diversity of existing agronomic practices, which choice will influence to a large extent the comparison with a selected GMHP-based management.

4.3.2 Gene therapy

- The spread of the GMO(s) in the environment
  Mechanisms for release of gene therapy products in the environment include dispersal during normal handling and use (*e.g.* wound leakage), accidental release and disposal of unused product, waste product and patient excreta (EMA, 2005). In contrast to GM plants for which the introduction in the environment is intrinsic to their deployment, the release of a gene therapy product is not the purpose, but a consequence of its primary use, *i.e.* the treatment of the patient.

  The route of administration, the potential for replication and biodistribution largely determine whether the gene therapy product ends up in the environment after administration. Material may be accidentally spilled at the moment that samples are taken. However, most attention has been given to "shedding", defined as the dissemination of the vector through secretions and/or excreta of the patient. After the therapeutic injection local shedding can be expected. When the vector is biodistributed through the blood vessels, shedding via urine, faeces and body fluids might be possible. Replication-competent viral vectors might persist in the patient for extended periods and can increase in amount. Therefore, the potential for shedding can be higher with replicating vectors and could result in a greater likelihood of transmission. Replication competent vectors can be applied in an environmentally safe way, in particular with adequate built-in safeties, such as conditional replication competency. Shedding also depends on the route of vector administration. The topics 'shedding' as a means of exposure, and 'replication competency' have received much attention (CGM 2010-10; RIVM, 2008a; Schenk-Braat *et al*., 2007; Van den Akker *et al*., 2013). Irrespective of the shedding potential, it is recognised that, although it is an important element in the ERA, in itself it does not automatically constitute an effect on the environment or human health.

- The transfer of the inserted genetic material to other organisms
  For recombination between the viral vector and wild-type viruses, both need to be simultaneously present in the same cell. *E.g.* this might occur when the treated person has an acute viral infection of a relevant type. An estimation of this possibility depends on the fate of the GMO in the patient's body and the infection route of the wild-type. Combining these elements may provide an insight in the possibility and frequency of assembly of new viruses or activation of latent viruses. Depending on the vector system, other mechanisms of exchange of genetic material may be applicable (*e.g.* conjugation and transduction for bacteria). As this possibility has been recognised, most vector systems have been designed to include features that prevent or reduce the likelihood of a successful exchange of genetic material.

- Phenotypic and genetic instability
  The stability of the intended trait remains a critical factor for determining the effectiveness and safety of the treatment. In addition, also the overall safety of the vector system must be guaranteed. As pointed out many vector systems have been derived from pathogenic organisms by attenuation and/or deletion of pathogenic functions. The stability of these features must be confirmed in order to avoid reversal to the wild-type or more virulent form. Given the diversity of vectors used so far, it seems too early to draw general conclusions.

- Interactions with other organisms
  The most important concern related to interactions seems the possibility for exchange of genetic information leading to gain of virulence functions and wild-type reversal. Bacteria may colonise the human gut and thereby replace local microflora. For a *Lactococcus* based expression system it was shown that the gene therapy products were readily evacuated from the gut system and that no colonisation occurred. However, given the diversity of vectors used so far, it is too early to draw general conclusions.

- Changes in management / treatment
  A comparison of the management of the GMO with the non-GMO counterpart is not relevant as the gene therapy treatment is completely new. Nevertheless, effects on/of other treatments (*e.g.* immune modulation, radiotherapy or chemotherapy) are addressed as they are sometimes combined in the study protocols.

4.4 Effect

In order to define the impact, protection goals must be explicitly stated. Furthermore in order to decide if an effect is acceptable or not, acceptance levels must be known. It was pointed out before that this for many aspects is not yet the case, hence the focus on certain areas of concern and the establishment of the variation already present today.

4.4.1 GMHP

- Biodiversity
  Several of the areas of concern (persistence and invasiveness in natural habitats, selective advantage or disadvantage in the GMHP and sexually compatible species, changed management practices) address the underlying concern that the introduction of the GMHP may influence plant biodiversity. Agriculture and agronomic practice as a whole have undoubtedly an important impact. Baseline studies are conducted to evaluate the level of variation relating to *e.g.* crop rotation, irrigation, use of plant protection products, etc.

  In the case of a herbicide tolerance trait, the target species are the weeds and botanical diversity is not affected by the GMHP, but potentially by the application of herbicides that are intended to manage weed problems. The GRACE systematic review question: “what are the effects of the cultivation of GM herbicide tolerant crops on botanical diversity?” investigated to what extent this is the case. The effects seem to depend on factors such as comparing herbicide treatments, exposed weed species, environmental and management factors, with glyphosate reducing weed populations more than glufosinate ammonium that showed a more variable picture. However, it must be borne in mind that these are effects of the herbicide application, not the genetic modification *per se*, and in consequence highly dependent on the actual application regime and used reference treatments.

  GMHP being integrated in agronomic practices will be part of this impact. Barfoot & Brookes (2014) conclude:

  > *GM traits have contributed to a significant reduction in the environmental impact associated with insecticide and herbicide use on the areas devoted to GM crops. Since 1996, the use of pesticides on the GM crop area was reduced by 503 million kg of active ingredient (8.8% reduction), and the environmental impact associated with herbicide and insecticide use on these crops, as measured by the Environmental Impact Quotient indicator, fell by 18.7%.*

  The challenge remains to develop efficient agricultural production systems that at the same time maintain or improve biodiversity at different levels. While GM technology offers additional options, it is a policy decision to explore if and how this can be achieved. In the studies conducted so far, no negative impact on biodiversity can be attributed to genetic modification.

- Target organisms
  The main concern related to target organisms has been the development of resistance that would reduce the efficacy of the introduced protection. The use of insect resistance genes may lead to selection of resistant insects and so promote resistance development in target insects. Management options are imposed to avoid or reduce the rate of resistance development that otherwise may become an environmental issue as other insecticides have to be utilised.

  The GRACE project examined the question: "How susceptible are different lepidopteran/ coleopteran maize pests to Bt proteins?" Most data are available for Cry1Ab. There is data variability and data availability is unequally distributed between species (most data are on *Helicoverpa armigera*).

  Cases of resistance selection in insects (for Bt crops) and weeds (for glyphosate on glyphosate tolerant crops) have been reported. This resulted in modifications of resistance management schemes.

- Non-target organisms
  Mostly linked to herbicide tolerance and insect resistance traits, NTO studies have been conducted. Tiered testing systems have been proposed as providing the most reliable results, compared with open-field observations. Research extends to several trophic levels and covers different ecosystem services. The recent consultation by EFSA[69] on "Guidance to define protection goals for environmental risk assessment in relation to biodiversity and ecosystem services" provides detailed indications on this matter.

  Plants form the first tier of food chains and therefore changes in weed populations influence other components of biodiversity, *i.e.* the NTOs important for pollination and pest control. The Farm Scale Evaluations already studied this topic. Again the relative effects on farmland wildlife varied according to the crop-herbicide combination and the comparative non-GM crop management.

  The GRACE consortium systematically examined the available literature using the review question: "Does the growing of Bt maize change abundance or ecological function of non-target animals compared to the growing of non-GM maize?" For maize no effect was found for vertebrates or off-crop situations. Non-target Lepidoptera studies were inconsistent. Within crop fields most effects were not significant when compared to untreated non-Bt maize. Compared with treated non-Bt maize significant positive effects were found. Also in cotton studies were performed with similar outcomes.

  It must be noted that Cry proteins are either naturally present or sprayed as a *B. thuringiensis* spore suspension. Microbial preparations of *B. thuringiensis* are currently approved and used in crops, especially in organic agriculture. However, routes of exposure and exposure levels are different.

  The GRACE systematic review question: "Are population abundances and biomasses of soil invertebrates changed by Bt crops compared with conventional crops?" did not reveal any significant effect on soil invertebrates. For soil micro-organisms the question was asked: "Are soil microbial endpoints changed by Bt crops compared with conventional crops?" The systematic review found no major effects (positive or negative) on microbial endpoints as compared to conventional crops. Minor short term effect may be visible but they are smaller compared to other influencing factors like the plant's growth stage, plant species and variety, the soil type, the year and the time of the year.

- Human and animal health
  This aspect covers potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s) and therefore differs from food and/or feed use. Human and animal health effects are studied analysing the crop's composition, the degradation in the digestive tract and potential for

[69] EFSA, Question No EFSA-Q-2013-00289

allergy development. Most GMHP evaluated so far had also been subject to a GM food and feed evaluation which already sets high standards for risk evaluation. For products approved so far no effects have been reported.

- Biogeochemical processes
  Biogeochemical impact assessments address essential functions such as decomposition and nutrient transformations. Many studies have been performed to construct a baseline and develop methods to monitor (ERGO project, CGM 2003-03). Given the fact that neither the main GM crops, nor the introduced traits contribute in a special way to the biogeochemical cycle, it is not unexpected that no effects have been observed.

Monitoring reports on commercialised crops in general confirm the results of the ERA. PMEM reports on the cultivation of Cry1Ab maize in the EU did not reveal any unanticipated effect. In GM adopting countries outside the EU in general very few unexpected effects were observed for herbicide tolerant and Bt crops (CGM 2010-08). Direct effects caused by the genetic modification were not found, only indirect effects with glyphosate tolerant crops: a reduced uptake of micro-nutrients and some positive and negative effects on susceptibility to diseases.

4.4.2 Gene therapy

- Biodiversity
  While aspects of persistence and invasiveness in natural habitats, and selective advantage or disadvantage of the gene therapy product and possible related organisms, must be addressed, the ERA is mostly related to human safety. The main concern is related to the pathogenic features (inherent or regained, from the vector system or from related organisms) that could lead to negative impacts on the patient and the population. However, given the diversity of applications so far, it seems too early to draw general conclusions.

- Target organisms
  In most cases, the patient (or specific cells of the patient) will be the “target” and patient safety remains the primary concern. Patient safety, starting with the first in man trial, is fully covered by the medicinal product legislation and the incumbent authorisation procedures.

  Integration of DNA might be desired or tolerated in target cells but should be minimised in non-target cells. It is of particular concern for gonadal tissue where, although unlikely, there exists the potential for modifying the germline. The risk of inadvertent germline integration is based on a number of factors including vector type, dose, route, and site of administration. For vectors that are unable to enter the target cell nucleus and remain cytoplasmic this risk is highly unlikely.

- Non-target organisms
  Other humans (staff, relatives, and offspring) are in principle NTOs. Their safety may already be (partly) included as an extension of patient safety. Protection of staff is handled via legislation on workers protection. The effect on relatives and the broad public remains to be considered and will largely depend on the introduced trait. Complicating factors such as uncertainty on immuno-competence, viral infection status, general health conditions, etc. will need to be taken into account. Aerts Kaya (2010) provides an example of an overview of how immune-modulation impacts and how it can be considered in an ERA.

- Human and animal health
  Human health is covered in the previous points. An effect on animal health would be a non-target effect *e.g.* when a pet or farm animal is in close contact with a treated patient. The likelihood depends on the presence of an infectious gene therapy product and its host range. The effect will then depend on the infectivity of the vector for that animal, tropism in the animal and the introduced traits.

- Biogeochemical processes
  This has not been identified as a relevant topic for gene therapy applications.

5 Translating remaining uncertainty in research priorities

The mandate of this report included the identification of priority areas for further research needed to address specific ERA challenges. From the preceding review of information, it was concluded that certain aspects have already been covered in detail and that risk assessors may find the information adequate or will be able to identify methods for assessing specific products. For other aspects of the ERA, the information may still be missing, scattered or too case-specific. This will be more prominent for new applications, although the available information can provide insights on how the safety demonstration can be tackled.

5.1 Identifying research priorities

Considering priorities implicitly requires an outlook on which applications will be presented in the coming years. In this context, the authors have made the following forward-looking assumptions:

- Cultivation of GM crops in the EU will remain predominantly limited to already approved crops and variations on known agronomic traits.
  In spite of EU field trials with different GM plant species, it is difficult to foresee a change in the political climate that would enable EU-wide commercial introduction of a large variety of species. The most likely crops to be cultivated in the EU will be maize, sugarbeet, soybean, cotton and potato. Similarly, it can be expected that the main traits will remain insect resistance and tolerance for specific herbicides, possibly combined with tolerance to specific diseases and drought.

  Even if gradually more diverse GM crops are introduced, it will require several years for developers to update their programmes for the EU. Possible extensions may also include novel solutions addressing disease challenges and yield enhancement that can only be offered by genetic modification.

- Import of GM crop products from non-EU production regions will increase and further diversify.
  Whereas so far most GM crops were initially introduced on the North-American market (USA and Canada), also products developed for and introduced in South America (Brazil), Asia (*e.g.* China, the Philippines, Bangladesh, India), Australia and Africa may be imported in the EU. This will broaden the type of products with products such as beans, cassava, banana, eucalyptus, pine apple and citrus. The type of characteristics at the same time expands to areas of disease resistance (bacterial, fungal, viral), pest resistance (nematodes, insects), yield enhancement and preservation (*e.g.* prolonged shelf life), abiotic stress tolerance (*e.g.* drought tolerance) and nutritional aspects (*e.g.* fortified foods).

  The ERA conducted by EU risk assessors will focus on imported products that are viable, most imported processed products solely being subject to the GM Food and Feed legislation. As demonstrated for import applications for GM maize, cotton, soybean and oilseed rape, an ERA will only be required to cover adverse effects from spillage of living material. While the information gathered for these products may be adequate for an ERA in the intended markets, for many of the smaller and less industrial developed crops it will be a hurdle to present data packages as demanded by EU regulation and supporting guidance.

- More gene therapy products will reach market introduction
  In contrast to a large number of GM plant applications, only few gene therapy applications have reached the commercial phase. However, with the first cases having established a path to the market, it should be clearer to evaluate for subsequent candidate products if this path is feasible. Consequently, more products can be expected to advance to a stage that requires an ERA. Given the development timelines and investments, the type of vectors and characteristics will be those already in the pipeline.

Patients can travel to countries for treatments not approved in the EU. In spite of legal frameworks established in such countries, it is not clear if their implementation will be performant enough to provide guarantees similar to the EU system. In addition to patient safety, this could have an effect on the environment and public health when the treated person returns to the EU. So far, medical tourism for gene therapy still seems to be limited and a combination of factors (costs, availability of treatments close-by) will remain a limiting factor.

The Standing Committee on Agricultural Research (SCAR)[70], more specifically the SCAR Collaborative Working Group Risk Research on Genetically Modified Organisms started in October 2009 to describe the status quo of risk research activities and knowledge, and to identify relevant research needs and gaps[71]. In their final report 'Priority Topics in Risk Research on Genetically Modified Organisms' (SCAR, 2012) the following topics regarding the environment were defined as areas where further research could be needed:

- Development and evaluation of methods for testing the environmental effects of novel GMOs (new species and/or new traits).
  So far, most ERAs for commercial release of GM plants relate to 'big' row crops such as maize, soybean, cotton and oilseed rape. Other plants, *e.g.* vegetable crops and (fruit) trees will require a different testing methodology. Similarly, new traits such as drought and salt tolerance and nutrient use efficiency might pose specific challenges in hypothesis testing.
- Development of methodologies for baseline data collection and use of these methods for the determination of the range of variation and analysis for different European receiving environments and of different agricultural practices and their environmental effects.
  The second topic calls for a refinement of a description of the receiving environment to reflect the range of environments and agricultural systems in the EU.

As part of the PreSto GMO ERA-Net project, stakeholders were asked to prioritise identified research needs. The following topics were considered to present information gaps relevant for an ERA for plants (organised according to different topics by the authors of this report):

- ERA methodology
  - Transfer of non-EU research on impacts to EU situation (environment)
- Baseline
  - Research into what constitutes a healthy soil as a precursor to being able to measure positive (and negative) effects of GM products (such as nitrogen use-efficient wheat, corn and other species)
- Impact on target organisms
  - How long does a GM-created resistance last before natural evolutionary processes in pests/fungi/bacteria "catch up" with it?
  - Does pollen-mediated gene transfer in outcrossing species increase the frequency of onset of HT weeds? Will the genes increasing tolerance to herbicide be transmitted via pollen into weedy species and if so will persistence of weeds increase?
- Impact on NTO
  - Impact of weed suppression (weeds as insect food) on pollination – in natural and for agricultural production, *e.g.* orchards
  - Impact of biofortified GM plants on non-target organisms that might consume them
- Impact of biodiversity
  - Effect of GM crops on biodiversity
  - Possible changes upon local ecosystems including plants, invertebrates and vertebrates, when species are dramatically changed
- Impact on ecosystem functions
  - Impact of GM plants on energy use and greenhouse gas emissions
  - Effect of GM forests on the forest ecosystem and effect on forest function
- Impact of management measures
  - Environmental effects of altered crop management due to use of GM crops

[70] http://ec.europa.eu/research/scar/index.cfm?pg=about

[71] http://bmg.gv.at/home/Schwerpunkte/Gentechnik/Fachinformation_Allgemeines/SCAR_Collaborative_Working_Group_Risk_Research_on_GMOs_

- Impact of (herbicide-tolerant and pest/disease-resistant) GM crops on agricultural practices

It must be considered that the ranking reflects priorities as perceived by different stakeholders and may reflect different aspects including a real information gap, lack of understanding the available information, different identification of protection goals, …

The authors are not aware of a similar effort to identify research priorities for gene therapy applications.

In line with the approach developed in the previous section, we organise the discussion based on the elements of the risk scenarios.

5.2 Stressor/activity

5.2.1 GMHP

The fact that the non-modified crops with a history of safe use can serve as reference in the comparison has been fundamental for the ERA of GMHPs. While this is well established for field crops, the methodology is not suited for all plants. *E.g.* trees in many ways represent different biological features (such as means of propagation, multi-season lifespans, and complex environmental interactions). The comparative testing methods used for field crops will not be applicable for trees albeit because of spatial and temporal dimensions.

Some developments of GM plants targeting non-food/non-feed uses have raised safety concerns. *E.g.* the production of pharmaceutical products in food crops may require stringent control of segregation. Still, there is no indication for short- or mid-term introduction of such applications as deliberate release in the EU.

It is expected that most GM plant products will retain traits like specific herbicide tolerances and insect pest resistances. Research on resistance development in the target populations remains of interest, but until there is a reasonable scale deployment in the EU, information will be limited to what is observed in other regions.

One area of concern addresses differences in invasiveness and/or persistence due to the genetic modification. While defining the characteristics that determine these features remains subject to further fundamental ecological research, it seems unlikely that any single trait will fundamentally change the biology of domesticated crop plants. This may have to be revisited for modifications addressing essential aspects of the life cycle of the GM plant (*e.g.* modification of the reproductive biology).

In addition to new traits, also new technologies are being introduced. An example is the use of RNAi. As it deviates from the central paradigm “DNA – RNA – protein/enzyme – function”, it presents uncertainty if and what type of information should be presented.

Finally, some traits have been “black-listed” in the EU or globally. Examples are:

- Marker genes:
  EU legislation incorporates the avoidance of the use of selection systems based on medically important antibiotics. In spite of detailed reviews and scientific opinions (*e.g*. from EFSA, 2004), confirming that the use of specific antibiotic resistance markers such as kanamycin resistance, will not exacerbate the threat to availability of effective antibiotics, it remains an argument in ERA related decision making.
- Genetic use restriction technology (GURT)
  GURT refers to technology that results in second generation seeds to be sterile. The harvested seed from this crop could not be used as seeds, but only for sale as food or fodder. The inability to produce seeds that can be replanted would force farmers to buy new planting material every season. The practice of farm-saved seeds, still predominant in developing countries, would therefor become impossible. For this reason, the United Nations Convention on Biological Diversity recommended in 2000 a *de facto* moratorium

on field testing and commercial sale of these so-called “terminator” seeds (CBD, 2000, COP5 Decision V/5 section III, paragraph 23). While the moratorium seems to allow continuation of farm-saved seed practice, it also blocks the possible benefits (*e.g.* improving volunteer management, avoiding establishment of the GM plants) in agronomic systems that do not rely on this practice.

These cases illustrate that in spite of advantages and safety, other policy-related factors play a role in decision making. In order to enhance the chance for new traits to be successful, it would be of interest to map such factors or, alternatively, identify circumstances under which even controversial technology can be deployed.

Recommended research topics:

- Adapting the comparative methodology for non-field crops (e.g. trees, fruits);
- Addressing crops with no or a diverging history of safe use (*e.g.* algae);
- Specifying requirements for the safety demonstration of non-protein based techniques (*e.g*. RNAi)

5.2.2 Gene therapy

When comparing different ERAs for gene therapy, concerns over negative effects originating from the vector are focussed on aspects such as altered pathogenicity, altered (cell) tropism, survival capacity, and altered replication capacity. While these considerations are clearly related to possible concerns, the underlying processes seem to be very diverse and case-specific. Furthermore the methodology to evaluate them may not be standardised. In many cases, observations in pre-clinical trials will be required to provide data supporting the ERA.

Similarly, little information is available on how some GM vectors perform outside of the target host. Susceptibility to disinfectants or sensitivity to prophylaxis are important features considering the need for a possible intervention following an unwanted release or exposure. Survival capacity extends to ex-host conditions such as sewage, dust, solid waste...

At the same time, the “environment” in most gene therapy ERAs is limited to human subjects (patients, staff, relatives and the population at large). While this reflects the intended target of the application (*i.e.* humans), it would be of interest to define criteria for deciding in which cases a broader environment (including animals, plants and/or micro-organisms) should be considered.

Recommended research topics:

- Description of specific features that influence pathogenicity, altered (cell) tropism, survival capacity, and altered replication capacity and options for testing;
- Identification of cases that require an ERA to address in more detail non-human environmental components.

5.3 Mechanisms

5.3.1 GMHP

While potential hybridisation with and introgression in sympatric compatible relatives is usually sufficiently documented, there may be cases in which the genetic modification intentionally would target changing the out-crossing and introgression capacity. A better understanding of which traits might have such an impact can support future ERA dealing with traits that influence the biology of the GMHP. This evaluation should also take into account that the success for introgression may be partly determined by any competitive advantage provided by the introduced trait. In this respect it is of interest to understand which traits are considered to be limiting the population of the compatible relatives.

Following the same rationale, it would be of interest to specifically investigate in which cases horizontal gene transfer could lead to an environmental concern *e.g.* taking into account the acquired character and the absence of similar traits in microbial communities.

Substantial documentation has been accumulated on the possible reaction of the target organisms as well as on the management of the development/selection of resistance in the insect population in relation to the application of Bt proteins, even more specifically Cry1Ab delta-endotoxin. However, many more Cry proteins as well as insect resistance traits based on other modes of action are available. GMHP combining different modes of action have been proposed as options for insect resistance management. The additional challenge that this involves different developers makes Insect Resistance Management an example of how information can be gathered and used. On the contrary, less is known on other "targeted" protections, *e.g.* when protecting plants against diseases (bacteria, fungi) or other pests (nematodes).

GM plants may interact with NTOs (other species associated with or sympatric with the GM plant) and this might result in adverse effects on biodiversity at several levels. For cases where target organisms have been identified, associated questions for potential impact on non-targets have been addressed. In applications with no evident target organism, this is addressed in more general terms. The definition of assessment endpoints remains an important challenge.

Experience with comparing management systems highlights how the diversity of existing agronomic practices impacts the comparison with a selected GMHP-based management. This variability influences the predictive nature for ERA since the effect will depend on what users eventually do and not on what was assumed/tested. Finally, the studies reveal a divergence of objectives: *e.g.* from an agronomic perspective efficient weed control may be preferred, whereas from an environmental viewpoint reduced species abundancy in a treated field may be seen as a negative impact. Rather than an *ex ante* evaluation, this aspect may be covered by an *ex ante* scenario development (*e.g.* in view of a specific protection goal, different integrated pest management strategies can be proposed) and an *ex post* collection of information.

Recommended research topics:

- Criteria for determining which characteristics would require a review of gene transfer to sexually compatible species;
- Criteria for determining which characteristics would require a review of horizontal gene transfer;
- Investigating the applicability of experience gained with early Bt proteins to other pest- and disease protection mechanisms;
- Criteria for determining when and what type of non-target studies are required;
- Scenario development for desired agricultural management changes.

5.3.2 Gene therapy

In most cases, the potential for transmission to untreated individuals via shedding is extremely low because of the derivation methods and/or modifications that are designed to attenuate the product compared to the parent strain. Still, vector shedding has received much attention as it represents a mechanism for release of gene therapy products in the environment. The evaluation of vector shedding is always a requirement for a phase I study (EMA, 2008). This can partly be attributed to the large diversity of vector designs and route of administration which influence shedding mechanisms. In spite of receiving much attention, heterogeneity in analysis methods and lack of documented findings during clinical trials do not allow yet to predict confidently the shedding potential for certain vectors. Acknowledging this heterogeneity, regulatory bodies have issued indications on how shedding studies should be designed (EMA, 2009; FDA 2015).

Irrespective, shedding is only the first step in exposure scenarios. Similar to considering "pollen flow" for plants, it is only one element in the ERA that needs to be combined with elements like the half-life in the patient and his immune response to the GMO (clearing of the GMO), persistence after shedding, susceptibility to environmental conditions (including use of disinfectants), possibilities for secondary infection of untreated persons, importance of the

immune status of the untreated person, etc. Schenk-Braat *et al.* (2006) stressed the uncertainty created by demonstrating that shedding occurs: it is in most cases unknown whether shed material -even if it contains infectious particles- can effectively result in an infection of a person and trigger clinical symptoms. FDA (2015b) suggests exploring other elements such as determining if shed material is infectious, comparing the amount of infectivity in the clinical samples to what is needed to initiate infection in a third party; and investigating whether the clinical sample containing the shed product represents a natural route of transmission. Monitoring of untreated individuals for transmission is not deemed very informative, because it is an extremely low probability event. Further documenting the complete risk scenario remains relevant for future ERA.

The concern over spreading for today's gene therapy products seems to be limited to other humans. It seems to be accepted that survival, colonisation and persistence of gene therapy products is only possible in humans. This might need to be revisited in case vectors are used with a different host range, infectivity and/or pathogenicity/virulence.

Depending on the type of vector, the integration of genetic material in the genome of the patient (or other exposed human) may be a concern. Genome homology and protection from degradation in the cell influence the probability. In case of viral sequences, it could lead to latent presence. A particular concern, although estimated to be extremely unlikely, under current conditions of use of gene therapy products, is the possibility for inadvertent germline transfer.

Risk associated with recombination and factors influencing the likelihood of recombination have been reviewed, *e.g.* Aerts Kaya (2010*)* surveyed the need to consider immune modulation in gene therapy ERA. Horizontal gene transfer to organisms present in the treated person or to organisms that are exposed upon release in the environment has received less attention so far. Irrespective of the transfer mechanism, the effect will largely depend on the introduced traits. In general, the potential for transfer to other organisms still has to be assessed case-by-case.

For other mechanisms, such as phenotypic and genotypic stability and interactions with other organisms, the case-by-case approach is also still required given the large diversity of vectors, inserted traits and type of applications.

Recommended research topics:

- Investigating what happens after shedding and which characteristics of a gene therapy product influence the likelihood that shed material results in an effect in untreated persons.
- Understanding mechanisms for horizontal gene transfer in the environment (*e.g.* bacterial vectors in sewage systems);

5.4 Effect

5.4.1 GMHP

- Biodiversity
  It remains a debate how and at what level biodiversity should be defined. Genetic diversity within the species is important to safeguard a gene pool for future breeding and adaption of the species to environmental challenges. Diversity of species in (semi-)natural habitats is an important nature conservation goal. Finally, at the habitat and landscape level, ecosystems represent intrinsic ecological webs.

- Target organisms
  Cases of resistance selection in insects (for Bt crops) and weeds (for glyphosate on glyphosate tolerant crops) have been reported. This has induced modifications of resistance management schemes. Although only relevant for areas with intense cultivation, this experience will be relevant at the time of an introduction in European growing areas. Some research is being conducted on baseline susceptibility and how to monitor resistance development.

- Non-target organisms
  In addition to baseline studies (*e.g.* the presence of arthropods in the EU – Meissle *et al.* 2012, Romeis *et al.* 2014), EFSA has indicated considering a more integrated ERA combining both the local and landscape scale, as well as the possible co-occurrence of multiple potential stressors and ecological recovery. The Scientific Committee[72] gathered scientific knowledge on the potential for recovery of NTOs for further development of ERA.

- Human and animal health
  For products approved so far no effects have been reported. The evaluation of such products usually coincided with a GM Food and Feed evaluation providing already a detailed argumentation on consumption related safety. Nevertheless, there remains a debate in which cases studies should be performed and which studies are adequate. As test systems for whole food testing have not been validated, the relevance and added value are questioned. Similarly, allergenic potential is based on indirect indications. Further improvements on methodology can strengthen both the ERA and food and feed evaluation for GMO as well as other products.

  Finally, production of specific products (*e.g.* plant-made pharmaceuticals) has been kept separated from the food/feed chain. The segregation challenges also motivate consideration of non-food/non-feed crops.

- Biogeochemical processes
  As most cases remained neutral so far in terms of contribution to biogeochemical processes, it will be necessary to consider what to do *e.g.* when traits like nitrogen use efficiency are envisaged.

Recommended research topics:

- Baseline studies on environmental protection goals remain of general relevance for a diversity of stressors (including anticipation of an introduction of GMHP);
- Rationalising toxicological and allergenicity testing for cases where no food and feed application is expected.

5.4.2 Gene therapy

- Biodiversity
  The main concern is related to the pathogenic features (inherent or regained, from the vector system or from related organisms) that could lead to negative impacts on the patient and the population. Depending on the ability to survive outside of the proper host and its duration, the GMO may impact biodiversity either by itself or by the expression of the introduced trait.

- Target organisms
  In most cases, the patient (or specific cells of the patient) will be the "target" and patient safety remains the primary concern. Patient safety, starting with the first in man trial, is fully covered by the medicinal product legislation and the incumbent authorisation procedures.

- Non-target organisms
  Other humans (staff, relatives, and offspring) are in principle NTOs. Their safety may already be (partly) included as an extension of patient safety. Protection of staff is handled via legislation on workers protection. The effect on relatives and the broad public remains to be considered and will largely depend on the introduced trait. Complicating factors such as uncertainty on immuno-competence, viral infection status, general health conditions, etc. will need to be taken into account. Critical reviews such as Aerts Kaya (2010) must be kept up-to-date as vectors evolve and more data become available.

- Human and animal health
  Human health is covered in the previous points. An effect on animal health will depend on the infectivity of the vector for that animal, tropism in the animal and the introduced traits.

[72] EFSA, Question No EFSA-Q-2013-00902

- Biogeochemical processes
  This has not been identified as relevant.

Recommended research topics:

- Determination of factors that may influence the virulence/ infectivity of selected gene therapy vectors;
- Survival and dissemination of the GMO in the environment;
- Maintaining an overview on the effect of complicating factors (immuno-compromised status, viral infection status, general health) on selected vector/trait combinations.

6 Conclusion

This report presents an overview of the type of information that is available to risk assessors when conducting an ERA for GMO. It ranges from independent studies to meta-analysis, from monographs to systematic reviews. More information is retrievable for GMHPs compared with gene therapy products. Yet, the question is not how much information is available, rather if the available information allows conducting an ERA with sufficient confidence.

The stepwise, case-by-case approach has been successfully applied. GMHPs can build on nearly 30 years of experience with field trials and 20 years of commercial release in very different environments. Only few gene therapy products have been introduced, but a large diversity has been evaluated in the framework of clinical trials. Although both developments incorporate gene technology, their implementation is fundamentally different. Genetic modification of plants has so far focussed on introducing traits in on-going plant breeding processes, essentially enhancing the genetic diversity of the crop gene pool. Building on crops with a history of safe use, the targeted modifications were relatively small and had no impact on the main characteristics of the crop. Gene therapy, on the other hand, holds the potential of offering completely new ways of "drug" delivery and treatment of diseases using mechanisms that in many cases are derived from pathogenic strains. While exact targeting and expression is important, concerns over the pathogenic origin of the vectors remain of primary importance.

For GMHPs, cultivation in open environments is fundamental to their application. Yet, applying the precautionary principle, uncertainty over the potential environmental impact led to formulate areas of concern. Based on the review in this report, it can be concluded:

- Similar areas of concern have been implemented in all GMHP related regulatory frameworks;
- So far no additional areas of concern have been identified on a cumulative hectarage of more than 1.8 billion commercial hectares;
- ERA and associated research have resulted in a careful approach to identify differences between GM and non-GM, to establish and test mechanisms through which an impact can occur and to determine the importance of that impact;
- Such information has further enabled to define and adapt management measures including integrated pest management approaches;
- New challenges include extending this approach to non-field crops (*e.g*. trees) and to non-agronomic traits;
- The most important challenge however remains to agree on (specific) protection goals and to determine levels of acceptance.

Gene therapy on the contrary has been mostly characterised by advancing in contained use environments. Only few legal frameworks require consideration of environmental aspects in early stages. EU Member States adopt different approaches differentiating between "contained use" and "deliberate release" and consequently provide different levels of attention for the ERA. Focussing on containment allows a streamlined R&D process with only limited investment in studies related to environmental concerns. Postponement of addressing the environmental concerns to a later development phase encourages research projects to proceed to a stage where results justify investment in a regulatory data package. Conversely, postponement may result in late identification of ERA issues that require additional efforts at a late stage of a development project. An early identification would allow better informed project choices as well as providing time to accumulate the necessary information.

Irrespective, experience with ERA can lead to streamlining of procedures. After dealing with multiple confined field trial applications, USDA-APHIS-BRS established the notification process for the introduction of genetically engineered plants meeting specific eligibility criteria as an administratively streamlined alternative to the permit process. Although the number of ERAs performed by European Member States is smaller, the accumulated information and experience should allow for a similar approach.

In principle the same areas of concern must be addressed in an ERA for GMHP and gene therapy. Yet, aspects related to *e.g.* biodiversity or effect on biogeochemical cycle is in most gene therapy

cases deemed less relevant. In contrast to GMHP, gene therapy seems to benefit from clarity on protection goals: the main (and often only) objective is human health relating to patient safety (covered by other legislation) and human safety in general (staff, relatives). Although it must be recognised that less information on environmental impact of gene therapy is available, much can be gained by further integrating the available information in risk scenarios. Individual findings (*e.g.* on shedding) may provide sufficient insight in part of a mechanism, but would only represent one step in a pathway to (potential) harm.

Although it can be expected that for certain GMOs information will continue to be accumulated, this will not automatically lead to an improved ERA, rather it will further strengthen the supporting information. In this respect it can be argued that further elaboration of test systems and regulatory requirements may only present a perception of a better ERA. More fundamental progress of ERA for GMOs as well as other stressors must be based on improved understanding of environmental interactions and on scientifically based problem formulation.

The experience gained so far confirms that GMOs are intrinsically neither more, nor less safe than other products. Taking stock of the ERA experience over 25 years can be a milestone in the discussion if the precautionary approach should specifically and solely be applied to organisms that are defined as “GMO”.

7 References

Aerts Kaya F.S.F., 2010, Immune modulation in gene therapy studies. Points to consider for Environmental Risk Assessment. 125pp.
http://repub.eur.nl/pub/21967/

Albajes R., Farinós G.P., Pérez-Hedo M., de la Poza M., Lumbierres B., Ortego F., Pons X., Castañera P., 2012, Post-market environmental monitoring of Bt maize in Spain: Non-target effects of varieties derived from the event MON810 on predatory fauna. Span J Agric Res 10(4), 977-985.

Anliker B.,·Longhurst S.,·Buchholz C.J., 2010, Environmental risk assessment for medicinal products containing genetically modified organisms. Bundesgesundheitsbl 53, 52–57.

Atkins Environment, 2008 Review of the environmental risks from marketing GM veterinary and human medicines. 158pp.
http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=15130

Barfoot P., Brookes G., 2014, Key global environmental impacts of genetically modified (GM) crop use 1996–2012, GM Crops & Food, 5:2, 149-160.

Bleijs D.A., 2005, Workshop on shedding data from gene therapy studies with viral vectors. Amsterdam, January 22, 2004. J Gene Med 7, 256–259.

CBD, 2000, COP 5 Decision V/5 Agricultural biological diversity: review of phase I of the programme of work and adoption of a multi-year work programme.
https://www.cbd.int/decision/cop/default.shtml?id=7147

CERA, 2011a, A review of the environmental safety of the CP4 EPSPS protein. Environ. Biosafety Res. 10: 5–25.

CERA, 2011b, A review of the environmental safety of the Cry1Ac protein. Environ. Biosafety Res. 10: 27–49.

CERA, 2011c, A review of the environmental safety of the Cry1Ab protein. Environ. Biosafety Res. 10: 51-71.

CERA, 2011d, A review of the environmental safety of the PAT protein. Environ. Biosafety Res. 10: 73–101

CERA,2013, A Review of the Environmental Safety of the Cry34Ab1 and Cry35Ab1 Proteins.
http://cera-gmc.org/files/cera/uploads/Cry3435_en_rev1.pdf

Clive J., 2014, Global Status of Commercialized Biotech/GM Crops: 2014. ISAAA Brief No. 49. ISAAA: Ithaca, NY.

COGEM Signalering CGM/141222-02, 2014, "Een uitstekend milieu: grenzen aan het milieu". 16pp.

CPB, 2012, Summary and Comparative Analysis of Nine National Approaches to Ecological Risk Assessment of Living Modified Organisms in the Context of the Cartagena Protocol on Biosafety. 178pp.
http://bch.cbd.int/protocol/cpb_technicalseries/cpb-ts-02-en.pdf

Craig W, Tepfer M, Degrassi G, and Ripandelli D., 2008. An overview of general features of risk assessments of genetically modified crops. Euphytica 164: 853-80.

Decision Document DD2013-99 Determination of the Safety of Monsanto Canada Inc.'s Soybean (*Glycine max* (L.) Merr.) Event MON 87712.
http://www.inspection.gc.ca/plants/plants-with-novel-traits/approved-under-review/decision-documents/dd-2013-99/eng/1414091707258/1414091707899

Dismuke D.J., Tenenbaum L., Samulski R.J. , 2013, Biosafety of recombinant adeno-associated virus vectors. Curr Gene Ther 13(6): 434-452.

Dolezel M., Heissenberger A., Gaugitsch H., 2005, Ecological effects of genetically modified maize with insect resistance and/or herbicide tolerance. Band 6/05, ISBN 3-900019-63-0, 70pp.
http://bmg.gv.at/home/Schwerpunkte/Gentechnik/Fachinformation_Gruene_Gentechnik/Studie_Oekologische_Effekte_von_gentechnisch_veraendertem_Mais_mit_Insektenresistenz_und_oder_Herbizidresistenz

EC, 2001, EC-sponsored research on safety of genetically modified organisms. A review of results. Luxembourg, ISBN: 92-894-1527-4, 246pp.

EC, 2010, A decade of EU-funded GMO research (2001 - 2010). Luxembourg, ISBN 978-92-79-16344-9, doi 10.2777/97784, 268pp.

EFSA, 2004, Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants, The EFSA Journal 48, 1-18.

EFSA, 2005, Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds, The EFSA Journal 181, 1-33.

EFSA (2008) Working Document of the GMO Panel on the interplay between Directive 2001/18/EC (GMOs) and Directive 91/414/EEC (Plant Protection Products). EFSA Panel on Genetically Modified Organisms (GMO), 8pp.
http://www.efsa.europa.eu/en/efsajournal/pub/1613

EFSA, 2009a, Scientific opinion on Guidance for the risk assessment of genetically modified plants used for non-food or non-feed purposes. The EFSA Journal 1164, 1-42.

EFSA, 2009b, Scientific report of EFSA prepared by the GMO Unit on Public Consultation on the Updated Guidance Document of the Scientific Panel on Genetically Modified Organisms (GMO) for the risk assessment of genetically modified plants and derived food and feed. The EFSA Scientific Report 293, 1-18.

EFSA, 2010a, Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 8(11):1879. 111pp.

EFSA, 2010b, Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 8(7):1700. 168 pp.

EFSA, 2010c, Scientific Opinion on the assessment of potential impacts of genetically modified plants on non-target organisms. EFSA Journal 8(11):1877. 75pp.

EFSA, 2011a, Guidance document on Selection of Comparators for the Risk Assessment of GM Plants. EFSA Journal 9(5):2149. 21pp.

EFSA, 2011b, Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants, EFSA Journal 2011;9(8):2316. 40pp.

EFSA, 2015a, Guidance on the agronomic and phenotypic characterisation of genetically modified plants. EFSA Journal 13(6):4128, 44 pp.

EFSA, 2015b, Guidance on Uncertainty in EFSA Scientific Assessment. EFSA Journal 20YY; volume(issue):NNNN, 219 pp.

EMA, 2005, Environmental Risk assessments for medicinal products containing, or consisting of Genetically Modified Organisms (GMOs) (Module 1.6.2) (EMEA/CHMP/BWP/135148/2004) 17pp.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003806.pdf

EMA, 2006, ICH Consideration - General Principles to address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors (CHMP/ICH/469991/2006) 4pp.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002679.pdf

EMA, 2007a, Guideline on Environmental Risk Assessments for Medicinal Products Containing, or Consisting of, Genetically Modified Organisms (GMOs) (Module 1.6.2) - Corr (EMEA/CHMP/BWP/473191/2006) 14pp.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003805.pdf

EMA, 2007b, Guideline on Scientific Requirements for the Environmental Risk Assessment of Gene Therapy Medicinal Products (EMEA/CHMP/GTWP/125491/2006) 14pp.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003744.pdf

EMA, 2009, ICH Consideration - General Principles to address Virus and Vector Shedding (EMEA/CHMP/ICH/449035/2009) 7pp.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002680.pdf

EMA, 2012a, Committee for Medicinal Products for Human Use (CHMP). Assessment Report Glybera. EMA/882900/2011. 147pp.
http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002145/WC500135476.pdf

EMA, 2012b, Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (EMA/CAT/GTWP/671639/2008) 17pp.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/05/WC500126836.pdf

EPA, 2010, BIOPESTICIDE REGISTRATION ACTION DOCUMENT *Bacillus thuringiensis* Cry1Ac Protein and the Genetic Material (Vector PV-GMIR9) Necessary for Its Production in MON 87701 (OECD Unique Identifier: MON 877Ø1-2) Soybean [PC Code 006532]. 39pp.

FDA, 1998, Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications. 42pp.
http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070561.pdf

FDA, 2015a, Guidance for Industry: Determining the Need for and Content of Environmental Assessments for Gene Therapies, Vectored Vaccines, and Related Recombinant Viral or Microbial Products. 14pp.
http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM439273.pdf

FDA, 2015b, Guidance for Industry: Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products. 19pp.
http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM404087.pdf

FERA, 2012, Environmental risks from research trials and marketing of genetically modified (GM) veterinary and human medicines. 60pp.
http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=17673

Garcia-Alonso M. and Raybould A., 2014, Protection goals in environmental risk assessment: a practical approach. Transgenic Res 23: 945–956.

Gonin P., Buchholz C.J., Pallardy M. and Mezzina M., 2005, Gene therapy bio-safety: scientific and regulatory issues. Gene Therapy 12, S146–S152.

Goossens M., Pauwels K., Willemarck N., Breyer D., 2013, Environmental risk assessment of clinical trials involving modified vaccinia virus Ankara (MVA)-based vectors. Curr Gene Ther. 13(6): 413-420.

Health Canada, 2012, Guidance for Industry: Preparation of Drug Submissions and Applications in the Common Technical Document (CTD) Format, June, 2012.
http://www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/ctd/ctd_prep_nds-eng.php

Henrys P.A., Thompson J., Smets G., Rüdelsheim P., Hails R., Freeman S., Glandorf D.C.M., Smith R., 2013, Review of statistical methods and data requirements to support post market environmental monitoring of agro ecosystems. Supporting Publications 2014:EN-582. 156 pp.
www.efsa.europa.eu/en/supporting/doc/3883ax1.pdf

HSE, 2007, The SACGM Compendium of guidance Part 6: Guidance on the use of genetically modified microorganisms in a clinical setting. 43pp.
http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part6.pdf

Johnson K.L., Raybould A.F., Hudson M.D., and Poppy G.M., 2007 How does scientific risk assessment fit within the wider risk analysis? Trends Plant Sci 12: 1-5.

Kok E.J., Pedersen J., Onori R., Sowa S., Schauzu M., De Schrijver A., Teeri T.H., 2014, Plants with stacked genetically modified events: to assess or not to assess? Trends in Biotechnology 32(2): 70-73.

Losey J.E., Rayor L.S. and Carter M.E., 1999. Transgenic pollen harms monarch larvae. Nature 399, 214.

Ma G., Shimada H., Hiroshima K., Tada Y., Suzuki N., Tagawa M., 2008, Gene medicine for cancer treatment: Commercially available medicine and accumulated clinical data in China. Drug Des Devel Ther. 2: 115–122.

MEA (2005) Millennium ecosystem assessment, ecosystem and human well-being: synthesis. Washington DC. 100pp.
http://www.millenniumassessment.org/en/index.html and
http://www.millenniumassessment.org/documents/document.354.aspx.pdf

Meissle M., Álvarez-Alfageme F., Malone L.A., Romeis J., 2012, Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU. Supporting Publications 2012: EN-334. European Food Safety Authority (EFSA), Parma. 170pp.
http://www.efsa.europa.eu/en/supporting/pub/334e.htm

National Academy of Sciences (NAS), 1989, Field testing genetically modified organisms. National Academy of Sciences, Washington, D.C. 170pp.

National Academy of Sciences (NAS), 1987, Introduction of recombinant DNA-engineered organisms into the environment: Key issues. National Academy of Sciences, Washington, D.C. 24pp.

OECD, 1986, Recombinant DNA Safety Considerations. 74pp.
http://www.oecd.org/env/ehs/biotrack/publicationsonharmonisationofregulatoryoversightinbiotechnology.htm

OECD, 1993, Safety considerations for biotechnology: scale-up of crop plants. 43pp.
http://www.oecd.org/env/ehs/biotrack/publicationsonharmonisationofregulatoryoversightinbiotechnology.htm

OGTR, 2013, Risk Analysis Framework. 139pp.
http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/raffinal5-toc

Pauwels K., Gijsbers R., Toelen J., Schambach A., Willard-Gallo K., Verheust C., Debyser Z., Herman P., 2009, State-of-the-art lentiviral vectors for research use: Risk assessment and biosafety recommendations. Current Gene Therapy 9(6): 459-474.

Peng Z., 2005, Review: Current Status of Gendicine in China: Recombinant Human Ad-p53 Agent for Treatment of Cancers. Human Gene Therapy 16: 1016–1027.

Perseus, 2006, Analysis of the applicability of the contained use legislation for clinical trials. 128pp.
http://www.genetherapynet.com/download/EU-analysis-Contained-use.pdf

Raybould A., 2006, Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environ. Biosafety Res. 5: 119–125.

Raybould A., 2010, Reducing uncertainty in regulatory decision-making for transgenic crops. More ecological research or clearer environmental risk assessment? GM Crops 1(1): 1-7.

Raybould A., Tuttle A., Shore S., Stone T., 2010, Environmental risk assessments for transgenic crops producing output trait enzymes. Transgenic Res 19: 595–609.

RIVM, 2008a, Environmental risk assessment of replication competent viral vectors in gene therapy trials (RIVM Report 601850001/2008) 115pp.

RIVM, 2008b, Effect of administration route on biodistribution and shedding of replication-deficient viral vectors used in gene therapy. A literature study (RIVM Report 320001001/2008) 244p.

Romeis J., Meissle M., Álvarez-Alfageme F., Bigler F., Bohan D.A., Devos Y., Malone L.A., Pons X., Rauschen S., 2014, Potential use of an arthropod database to support the non-target risk assessment and monitoring of transgenic plants. Transgenic Res 23, 995–1013.

Rothe M., Modlich U., Schambach A., 2013, Biosafety Challenges for Use of Lentiviral Vectors in Gene Therapy. Curr Gene Ther. 13(6): 453-468.

SCAR, 2012, SCAR Collaborative Working Group Risk Research on Genetically Modified Organisms - Priority Topics in Risk Research on Genetically Modified Organisms. 2pp.
http://bmg.gv.at/home/Schwerpunkte/Gentechnik/Fachinformation_Allgemeines/SCAR_Collaborative_Working_Group_Risk_Research_on_GMOs_

SCBD, 2012, Guidance on Risk Assessment of Living Modified Organisms. Final Report of the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety, UNEP/CBD/BS/AHTEG-RA&RM/4/6.
http://bch.cbd.int/protocol/meetings/documents.shtml?eventid=5037 (last accessed September 2015).

Schenk-Braat E.A., van Mierlo M.M., Wagemaker G., Bangma C.H., Kaptein L.C., 2007, An inventory of shedding data from clinical gene therapy trials. J Gene Med. 9(10): 910-921.

Sears M.K., Hellmich R.L., Stanley-Horn D.E., Oberhauser K.S., Pleasants J.M., Mattila H.R., Siegfried B.D., Dively G.P., 2001, Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. PNAS 98(21),11937-11942.

Spanish Ministry of Environment, 2003, Assessment report: notification number C/ES/01/01 to market genetically modified maize (line 1507) resistant to Lepidopterae and tolerant to glufosinate-ammonium herbicide submitted by the company Pioneer, in accordance with Directive 2001/18/EC. 7pp.

Spök A., Dolezel M., Eckerstorfer M., Freigassner M., Gaugitsch H., Heissenberger A., Karner S., Klade M., Proksch M., Schneider L., Treiber F., Uhl M., 2007, Assessment of toxic and ecotoxic properties of novel proteins in GMOs, Forschungsberichte der Sektion IV, Band 1/2008. 193pp.

Tepfer M., Jacquemond M., García-Arenal F., 2015, A critical evaluation of whether recombination in virus-resistant transgenic plants will lead to the emergence of novel viral diseases. New Phytol. 207(3), 536-541.

USDA-APHIS, 1996, Guide for Preparing and Submitting a Petition for Genetically Engineered Plants 36pp.

USDA-APHIS, 2012, Molecular Characterization for Transgenic Plants Intended for Unconfined Release; 8pp.

USDA-APHIS, undated, Environmental Characterization Data for Transgenic Plants Intended for Unconfined Release. 9pp.

USDA-APHIS, 2012a, Plant Pest Risk Assessment of Event H7-1 Sugar Beet. 17pp.
http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml

USDA-APHIS, 2012b, Final Environmental Impact Statement: Glyphosate-Tolerant H7-1 Sugar Beet. 801pp.
http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml

USDA-APHIS, 2014, Final Environmental Assessment: Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise (Simplot Innate™ potato). 113pp.
http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml

USDA FAS-GAIN, 2015, Amendments to China's GMO Safety Assessment Regulations. Report number CH15021. 16pp.

Van den Akker E., van der Vlugt C.J., Bleijs D.A., Bergmans H.E., 2013, Environmental risk assessment of replication competent viral vectors in gene therapy trials: potential effects of inserted sequences. Curr Gene Ther. 13(6): 395-412.

Verheust C., Goossens M., Pauwels K., Breyer D., 2012, Biosafety aspects of Modified Vaccinia Virus Ankara (MVA)-based vectors used for gene therapy or vaccination. Vaccine 30(16):2623-2632.

Wold W.S.M. and Toth K., 2013, Adenovirus Vectors for Gene Therapy, Vaccination and Cancer Gene Therapy. Curr Gene Ther. 13(6): 421-433.

Annex 1 Examples of the implementation of the ERA framework for GMOs in EU Member States

Member State	Legislation	Competent authority	Scientific advisory committee	Clinical trials with GMOs
The Netherlands	Directive 2001/18/EC is implemented in Dutch legislation as the '*Besluit genetisch gemodificeerde organismen milieubeheer 2013*' ('*Besluit GGO*') of the 1st of April 2014 (Staatsblad 2014, nr.157, 30.04.2014.). Art. 3.6 and 3.7 of the law requires an applicant to perform a risk analysis before starting a deliberate release in conformity with the European Directive. Concerning the details of the ERA the law refers to directive 2001/18/EC (Art. 1.5). Art. 7.2 clarifies the concept of an ERA. The implementing decree, '*Regeling genetisch gemodificeerde organismen milieubeheer 2013*' ('*Regeling GGO*') of the 14th of April 2014 (Staatscourant 2014, Nr. 11317, 30.04.2014) does not further elaborate on the ERA for a deliberate release. The application forms for deliberate releases of GMOs summarize the areas of concern in the conclusion part (A6) as described in Annex II part D.1 or D.2 of Directive 2001/18/EC.	The Ministry responsible for environment	Commission on Genetic Modification (COGEM)	Clinical trials with GMOs on humans are considered to be deliberate release trials
Belgium	The Belgian legislation on deliberate release of GMOs is the 'Koninklijk Besluit van 21 februari 2005 tot reglementering van de doelbewuste introductie in het leefmilieu evenals van het in de handel brengen van genetisch gemodificeerde organismen of van producten die er bevatten. (Belgisch Staatsblad 24.02.2005)'. Annexes II and III of this Decree translate Annexes II and III of Directive 2001/18.	The Ministries of Public Health and Environment	'Adviesraad voor Bioveiligheid (BAC)'	For clinical trials in all cases an authorisation must be obtained according to the contained use legislation. In the case of multicentre trials and/or for those trials that involve ambulatory medicine and the risk of excretion of GMOs by the patient into the environment the deliberate release legislation also needs to be followed.
France	The French Environmental Code gives the basis for the GMO regulations (Art. L. 124-3, Art. L. 531-1 et s., Art. L. 532-1 et s., Art. L. 533-1 et s., Art. L. 534-1, Art. L. 535-1 et s., Art. L. 536-1 et s., Art. L. 537-1, Art. D. 531-1 et s., Art. R. 532-1 et s., Art. R. 533-1 et s., Art. R. 536-1 et s.) and deals with the deliberate release of GMOs. Concerning the ERA (Article L533-3-1; Article L533-5) no specifications are made. The ERAs (Article L531-3). The Code itself does not give details but refers to community law and international guidance. Its advices also include benefits next to risks (Article L531-4). For non-commercial applications the 'Décret no 2007-358 du 19 mars 2007 relatif à la dissémination volontaire à toute autre fin que la mise sur le marché de produits composés en tout ou partie d'organismes génétiquement modifiés' refers to Annex II and III of Directive 2001/18.	Ministries of Environment, Agriculture, Research, Health and Consumer Affairs	'Haut conseil des biotechnologies'	Depending on the potential for releasing the GMO in the environment, clinical trials are handled as contained use or deliberate release activities.
Germany	The Genetic Engineering Act (Gentechnikgesetz - GenTG), regulates all handling with GMOs with the exception of the use of GMOs on humans. Art. 6.1 refers to the ERA that is required. The ‚Verordnung über Antrags- und Anmeldeunterlagen und über Genehmigungs- und Anmeldeverfahren nach dem Gentechnikgesetz (Gentechnik-Verfahrensverordnung - GenTVfV)' refers to Directive 2001/18 for details on the ERA (Section 2 §5.4). The German Drug Law (Arzneimittelgesetz, AMG) of 11th of December 1998 (BGBl. I S. 3586) and amendments, more in particular the 12th amendment (12.	The Federal Office of Consumers Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)) Paul-Ehrlich-Institut,	Central Commission on Biological Safety	

	AMG-Novelle) of 30th of July 2004 (enforcement: 6th of August 2004) regulate clinical trials. The 12th amendment is the transposition of the Directive 2001/20/EC[73] on clinical trials and integrates requirements of Directive 2001/18/EC on risk assessment.	residing with the Ministry of Health (Bundesministerium für Gesundheit).		
Spain	The 'LEY 9/2003, de 25 de abril, por la que se establece el régimen jurídico de la utilización confinada, liberación voluntaria y comercialización de organismos modificados genéticamente' is the basic law that regulates contained use and deliberate release of GMOs. The implementing law 'REAL DECRETO 178/2004, de 30 de enero, por el que se aprueba el Reglamento general para el desarrollo y ejecución de la Ley 9/2003, de 25 de abril, por la que se establece el régimen jurídico de la utilización confinada, liberación voluntaria y comercialización de organismos modificados genéticamente' lists the areas of concern to be taken into account in the ERA in Annex IV. They are the exact translation of the areas of concern in Annex II, D.1 and D.2 of Directive 2001/18.	Ministry of Environment.	The Spanish Biosafety Commission, the authorities of the autonomous regions and the inter-ministerial advisory committee discuss the ERA.	Clinical trials can be done as contained use or deliberate release trials. The particular conditions of each trial depend on the GMO and previous experience from animal or humans studies regarding biodistribution and persistence of the GMO. An authorisation is needed by the Autonomous Regions, the Agency for Medicines and Medical Devices and the Ministry of Environment.
UK	The Genetically Modified Organisms (Deliberate Release) Regulations 2002 (GMO(DR)) is the transposition of Directive 2001/18/EC and came into force on October 17, 2002. Part I Regulation 6 requires an ERA to be carried out according to Annex II, D of Directive 2001/18, further detailed in Schedules 1, 2 and 4. The essential difference between the contained use and deliberate release regulations is whether there is intention to release a GMO or if the action is expected to cause a GMO to be eventually released into the environment. This depends on the GMO's characteristics, such as: replication ability, attenuation level, possibility of shedding, survival outside. Rigorous ERAs are required for all "contained use" and "deliberate release" activities. Clearly, if the GMO is to be released into the environment under "deliberate release" regulations, then a far more detailed ERA will be requested.	The Health and Safety Executive (HSE) has the lead responsibility for regulation of the safety (to humans and the environment) aspects of activities involving GMOs in containment. The Department of the Environment, Food and Rural Affairs (DEFRA) and the Scottish Executive are joint competent authorities, scrutinizing the deliberate release notifications, with HSE joint competent authority covering risks to human health. Enforcement of the Contained Use Regulations is only dealt with by HSE.		Clinical trials are regarded either as contained use or as deliberate release applications. The Genetically Modified Organisms (Contained Use) Regulations 2014 (GMO(CU); SI 2014/1663 The Stationery Office), in force since the 1st of October 2014, require an ERA (Regulation 5) according to Schedule 3 (micro-organisms) or Schedule 6 (larger organisms). All gene therapy projects so far have been contained uses. A small number of vaccine trials have been deliberate releases. Some applicants have preferred to deal with the trial as a deliberate release (e.g. trials with vaccines). Firstly, this covers any possible introduction, although maybe not intended. Secondly, it allows for understanding the risk assessment that needs to be prepared by the time a Market Authorization may be sought. The main disadvantages seem to be the more complex procedure as well as the different level of confidentiality

[73] Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. OJ L121, 1.5.2001, p.34-44.

Annex 2 Comparison of areas of concern in selected countries

Table 1 Areas of concern in ERAs for higher plants as identified in EU (DIR 2001/18 and included in EU national implementations such as Dutch "Besluit GGO" 2014); Australia (basd on The Gene Technology Act 2000 and information from OGTR, 2013, Risk Analysis Framework); Canada (Dir94-08); People's Republic of China (Guideline for biosafety assessment); USA (7 CFR part 340 (PPRA) from decision documents); USA (40 CFR Parts 152 and 174 (FIFRA-PIP) from decision documents) and USA (40 CFR Parts 1500 through 1508 (NEPA) EA or EIS from decision documents)

EU	Australia	Canada	China	USA (PPRA)	USA (FIFRA-PIP)	USA (NEPA)
Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.	X	X	X	X	X	X
Any selective advantage or disadvantage conferred to the GMHP.	X	-	-	-	-	-
Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.	X	X	X	X	X + horizontal gene transfer	X
Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).	-	-	-	-	-	-
Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.	X	X	X	X	X	X
Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).	X	X	X	-	X	X
Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if	X	X	X	-	X	X

EU	Australia	Canada	China	USA (PPRA)	USA (FIFRA-PIP)	USA (NEPA)
it is intended to be used as animal feed.						
Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).	-	-	-	-	-	X
Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.	X	-	-	X	X	X
		Potential for the PNT/GMO to become a plant pest		Potential for the PNT/GMO to become a plant pest		
				Potential for the GMO to have altered disease and pest susceptibilities		
	Potential impact on biodiversity/ecosystem	Potential impact on biodiversity/ecosystem				Potential impact on biodiversity/ ecosystem
						Potential impact on the abiotic environment (air, soil, water)
Stacked events: Yes	no, unless new risks are identified			no, if parent events are already assessed	Only potential interaction of PIPs	

Table 2 Areas of concern in ERAs for organisms other than higher plants as identified in EU (DIR 2001/18 and included in EU national implementations such as Dutch "Besluit GGO" 2014); Australia (basd on The Gene Technology Act 2000 and information from OGTR, 2013, Risk Analysis Framework); Canada (CEPA - NSNR organisms); People's Republic of China ("Points to Consider for Human Gene Therapy and Product Quality Control"); USA (40 CFR Parts 1500 through 1508 (NEPA) EA or EIS from guidance FDA, 2015a) and USA (Occupational Safety and Health Act)

EU	Australia	Canada	China	USA (NEPA)	USA (OSHA)
Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).	X	X	X	X	-
Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).	X	X	-	X	-
Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.	X	X	X	X	-
Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).	-	-	-	-	-
Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.	X	X	X	X	-
Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).	X	X	X	-	X
Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.	X	-	-	-	-
Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).	-	X	-	-	-

EU	Australia	Canada	China	USA (NEPA)	USA (OSHA)
Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.	-	-	-	-	-
	Impact on biodiversity/ ecosystem	Protect the environment, including its biological diversity, and human health, by ensuring the safe and effective use of biotechnology			

Annex 3 Research commissioned or sponsored by Governmental bodies

Table 1 EFSA commissioned studies and organised events relative to ERA for plants

EFSA studies
Scientific Opinion on the use of existing environmental surveillance networks to support the post-market environmental monitoring of genetically modified plants Post-Market Environmental Monitoring (PMEM) of agro ecosystems (OC/EFSA/SAS/2012/02) (Henrys *et al*., 2014) The report shows how data obtained from existing monitoring networks and programmes may be used in PMEM.
Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU[74] (Meissle *et al*., 2014) Potential use of an arthropod database to support the non-target risk assessment and monitoring of transgenic plants (Romeis *et al.*, 2014). The database provides a detailed overview of the arthropod fauna in arable crops across Europe. It includes 3030 species and 14.762 abundance records from 31 European countries. Data originate from fields planted with maize, oilseed rape, potato, sugar/fodder beet, soybean, cotton, and rice, and in field margins. Arthropods in the database represent 278 families and 30 orders, with beetles (Coleoptera), aphids, bugs, and leafhoppers (Hemiptera), and spiders (Araneae) having the highest number of species and records. Few data on soil arthropods are recorded. The database can facilitate the identification of ecologically and agronomically relevant species for the assessment of potential adverse effects of GM crops on non-target arthropods. The related publication by Romeis *et al.* (2014) explains the NTA selecting steps for ERA: 1. Identify the ecosystem services 2. Identify the main taxonomic groups and/or species associated with the functional groups identified in Step 1 (identification of assessment endpoints). 3. Identify and prioritise NTA species from the lists built in Step 2 based on ecological criteria and the likely sensitivity to the insecticidal compound expressed in the GM plant 4. Assess whether the hypotheses formulated in Step 3 can be tested with existing data or whether additional NTA data are required. Furthermore, the use of the database is exemplified with hypothetical cases.
Strategy support for the Post-Market Monitoring (PMM) of GM plants: Review of existing PMM strategies developed for the safety assessment of human and animal health[75] The report provides a systematic review of existing monitoring programmes for food, feed, human and animal health and an inventory of data collection sources that may be useful for PMM of GM food and feed. The report also pinpoints to the hurdles in gathering data and makes recommendations for improvements.
In vitro digestibility tests for allergenicity assessment[76] The literature review identified a lack of harmonised test conditions and discusses ways to improve the test in order to make comparison of test results easier.
Non-IgE-mediated immune adverse reactions to foods[77] In this literature review adverse reactions to foods has been analysed and ranked with regards to the quality of evidence that the conditions are (1) triggered by foods and (2) have an immunological mechanism which is distinct from IgE-mediated food allergies. The sequences in gluten that are responsible for Coeliac disease and the associated conditions dermatitis hepertiformis and gluten ataxia have been identified making it possible to assess whether a newly expressed protein contains coeliac-toxic motifs. This could be incorporated into the current allergenicity risk assessment.

[74] http://www.efsa.europa.eu/en/supporting/pub/334e
[75] http://www.efsa.europa.eu/en/supporting/pub/739e
[76] http://www.efsa.europa.eu/en/supporting/pub/529e
[77] http://www.efsa.europa.eu/en/supporting/pub/527e

Review of the strategies for the comprehensive food and feed safety and nutritional assessment of GM plants per se[78] This systematic review looks at cases with new traits where the comparative approach may not be fully applicable to assess food and feed safety. Nevertheless, the authors found that in the scientific literature and with assessment bodies always a comparison was made, although a flexible approach may be required.
EFSA events
EFSA Scientific Colloquium N°19: Biodiversity as protection goal in environmental risk assessment for EU agro-ecosystems[79] The event was organized to support EFSA's work on specifying protection goals that can be applied to an agro-landscape regardless of the product (*e.g.* pesticides) or organism (*e.g*. GMOs) that is being assessed.
International scientific workshop: Risk assessment considerations for RNAi-based GM plants[80] The biology of RNAi, current and future applications of RNAi-based GM plants, and issues specific to their risk assessment were discussed
International scientific workshop: 'Non-target organisms and GM crops: Assessing the effects of Bt proteins'[81] This workshop, organised together with COGEM, discussed the latest scientific insights on Bt proteins, the selection of non-target organisms, modelling and the experimental design of laboratory and field experiments, in the context of ERA.

Table 2 Studies commissioned by authorities in the Netherlands relative to ERA for plants

the Netherlands Ministry of Environment
General Surveillance of genetically modified plants: Possibilities for implementation in the Netherlands (RIVM Report 601040001/2012) (Glandorf, 2012) The study discusses the possibility for the Ecological Monitoring Network that follows the development of flora and fauna in the Netherlands, and the Biological Indicator System of Soil Quality that keeps track of soil quality to be used for the general surveillance of genetically modified crops in the Netherlands.
Environmental risk assessment of proteins expressed by genetically modified plant: Applicability of standard tests used for chemical pesticides. (RIVM Report 601787002) (Scheepmaker JWA, 2010) The report argues the possible use of standard test for assessing chemical plant protection products to analyse the effect of newly expressed proteins in GM plants. Three cases are explored.
COGEM studies
Effects of genetically modified plants on soil ecosystems (2002) The report identified soil functions or groups of micro-organisms that are most relevant for determining and monitoring effects of GMPs on terrestrial ecosystems. An inventory was made of knowledge and the most suitable techniques to monitor that were available at the time of writing.
Knowledge gaps with respect to the effects of genetically modified crops on the functioning of soil ecosystems (CGM 2003-03) Using questionnaires and interviews an inventory of needs and knowledge gaps is presented with respect to the effects of genetically modified (GM) plants on soil ecosystems. Future research should study the baseline variation as a result of seasons, weather patterns and varying agricultural practices. Tools to test for GM crop-induced effects should be developed thereby standardising and streamlining methods.
Hybridisation and introgression between crops and wild relatives (CGM 2003-02)

[78] http://www.efsa.europa.eu/en/supporting/pub/480e
[79] http://www.efsa.europa.eu/en/events/event/131127
[80] http://www.efsa.europa.eu/en/events/event/140604
[81] http://www.efsa.europa.eu/en/events/event/121129

Hybrid formation and introgression are found between crops and wild relatives and is well documented. On the effects of hybridisation and introgression information is lacking especially fitness effects of transgenes. The report furthermore identifies a number of lacunas in our knowledge of introgression and its consequences.

Transfer of DNA from genetically modified plants to bacteria (CGM 2005-02)

A literature review and workshop results are presented on the persistence of transgenic plant DNA in the environment and on bacteria capturing DNA in the environment. Naturally-competent bacteria occur especially on the surface of soil particles, the rhizosphere, the within-plant environment, but also in the gastrointestinal tract of soil arthropods and of animals/humans. The presence of sequences in a transgenic plant with homology to sequences in bacterial genomes is a key factor for successful transfer. Prokaryotic-like expression signals are needed for expression.

Pollen viability in the field (CGM 2005-05)

The literature review found that factors that influence pollen viability are mainly drought/dehydration, heat stress and UV-B radiation. Field experiments include Brassica, strawberry and wheat. For Brassica, it was found that pollen retains some viability for at least 72 hours. In strawberry pollen viability drops faster: after 6 hours, strawberry pollen had lost approximately 50% of its viability. For wheat no conclusions could be drawn. The authors recommended that out crossing rates should be directly measured instead of via pollen viability.

Effects of insect-resistant transgenic crops on non-target arthropods: first step in premarket risk assessment (CGM2005-06)

Based on the scientific literature, ecological food webs have been constructed for maize potato and oilseed rape, representing the most relevant arthropod taxa/species and their interactions per crop. A total of 18 NTA species have been selected suitable for ERA.

Project inventarisatie frequentie van kruisbestuiving in plantensoorten die bekend staan als zelfbestuivers en apomicten (CGM 2007-06)

The report gives an overview of the present knowledge of outcrossing frequencies in a selection of the most relevant selfing or apomictic species. All of them are capable of outcrossing. Outcrossing rates vary with genotype, experimental design, pollinating agent, and other environmental conditions, particularly, stress conditions. This has led to revised requirements for containment in greenhouses.

Designing experimental protocols to investigate the impact of GM crops on non-target arthropods (CGM 2008-01)

Not only mortality but also sublethal effects like changes in development time or fecundity have to be taken into account to assess NTA impacts. The report presents detailed experimental protocols based on taking census of the population at different time points.

Inventory of observed unexpected environmental effects of genetically modified crops (CGM 2010-08)

The inventory was made of agronomic effects on the crops maize, sugar beet, potato, oilseed rape, alfalfa, soybean and cotton grown primarily in the USA. No major unexpected adverse effects were found. Only in glyphosate tolerant maize and soybean micro-nutrient uptake is sometimes decreased and disease susceptibility increased due to the application of the herbicide, not because of the transgene. Extra fertilizer and spray against disease could hev a negative effect on the environment.

The use of statistical tools in field testing for effects of GM plants on non-target organisms (NTOs) (CGM 2012-06)

The report evaluates various statistical models, their strengths and weaknesses, that are commonly used for field trials. A checklist is presented that is useful to assess whether field trial data were analysed correctly.

Inventory of possible crop cultivation changes as a result of the introduction of GM crops in the Maritime zone of Europe: An overview for Maize, Sugar beet and Potato (CGM 2012-09)

The authors evaluated changes in crop management of maize, sugarbeet and potatoes in the US after introduction of GM crops as they may be indicative for potential changes in Europe. Most of the changes are related to the introduced trait: no till practice for herbicide tolerant crops, changes in pesticide use, development of resistant weeds and insects.

Genetically modified oilseed rape (*Brassica napus*): Aspects in relation to the environmental risk assessment and post-market environmental monitoring of import applications (CGM/130402-01)

This advisory report describes the potential of oilseed rape to cross with wild relatives and to become feral along transport routes. Stacking of traits has been observed. This may result in a potentially adverse effect. The authors advice to make a general surveillance plan mandatory for feral populations in import dossiers of GM oilseed rape.

Inventory and guidelines for studies on the interactions of the soil microbiota with genetically modified (GM) plants (CGM 2013-03)

The report provides an overview of the available methods and techniques to study soil microbiota with their pros and cons. The authors describe how they may inform the ERA and how they can be used in a tiered approach.

The contribution of metabolomics research to the environmental risk assessment of genetically modified plants (CGM 2013-04)

This report reviews the current status of the methodologies used for the analysis of the soil microbiota, that are crucial for specific life support functions of soil, and then proposes guidance for the use of such methods in risk assessment. A set of examples illustrates the approach.

Bacillus thuringiensis toxins: their mode of action and the potential for interaction between them (CGM 2014-02)

The biochemical and toxicological characteristics of several Bt toxins are reported and a literature review of potential interactions between them. From these data it is observed that synergism may occur between 2 toxins but that this is dependent on the species that is tested. Knowledge gaps are identified, *e.g.* data on synergy in less sensitive non-target species.

Can interactions between Bt proteins be predicted? (CGM 2014-05)

The investigations found out that activity of Bt-toxins is not as specific as generally assumed. On the predictability it was found that:

- synergistic or antagonistic interactions are likely to occur when a Cyt protein is present;
- specificity of the Bt proteins (including primary order and cross-order specificity), is a useful tool in predicting if interactions might occur;
- if the specificities of Bt proteins do not overlap, the possibility that interactions will occur is unlikely;
- for Bt proteins with the same specificity, the current knowledge of interactions between Bt proteins is not sufficient to make a prediction; and
- the preliminary observation that interactions among Bt protein combinations can be synergistic for one insect species, but be neutral or antagonistic for another, complicates the predictability of the occurrence of interactions.

Ecological and experimental constraints for field trials to study potential effects of transgenic Bt-crops on non-target insects and spiders (CGM 2014-06)

The authors observed that field trials are only able to demonstrate large effects on NTAs. Small effects or effects on organisms that are present in low numbers, are mobile or where abundance is largely fluctuating cannot be detected. The report furthermore analysed the methods of sampling and field trial design that are currently used, their strengths and weaknesses. A suggestion is made to overcome the drawbacks mentioned above.

Inventariserend onderzoek naar de potentiële blootstelling van aquatische organismen aan plantmateriaal van Bt-Mais (CGM 2015-02)

As maize in the Netherlands is harvested in whole, maize plant material reaching water courses is mainly pollen. Research of the water surface, water column and sediment revealed that most pollen stay floating on the surface. When in the water column it lyses and the Bt toxin degrade. Accumulation in the sediment occurs. Calculations resulted in concentrations a factor 1,000,000 lower than toxin concentrations at which negative effects have been reported in literature. Based on these data no negative effects on aquatic organisms are expected.

COGEM events

Symposium report. Non-target organisms and GM crops: Assessing the effects of Bt proteins

(CGM/130912-01) COGEM and EFSA jointly organised this international scientific workshop on 29 and 30 November 2012. The report provides an overview of the presentations given, remarks made and the discussion points put forward during the workshop.
Event report International scientific workshop 'Stacked Bt genes: assessment of effects on non-target organisms' (CGM/141217-01) This workshop, organised together with EFSA, discussed the latest scientific insights on Bt proteins, the selection of non-target organisms, modelling and the experimental design of laboratory and field experiments, in the context of ERA.

Table 3 Research projects sponsored by the EU relative to ERA for plants

The following information is retrieved from EC, 2001, EC, 2010 and the Cordis website[82]

FP1 – BAP Biodiversity Action Plan research programme (EEC) (1984-1988)
Studies of interspecific gene transfer, maintenance and expression in nature (BAP-0422/0475/0476/0483/0486)(1988-1991)
Study of gene dispersal from plants produced by recombinant DNA technology (BAP-0371/0384/0408/0423)(1989-1990) Studies on potato, alfalfa, oilseed rape cross-pollination with the same (oilseed rape) or related weedy species (all crops); gene transfer is possible for alfalfa to *Medicago sativa* spp. *falcata*, *M. sativa* spp. *coerulea*, *M. sativa* spp. *glutinosa*, *M. saxatilis* and *M. cancellat*. Potato could not cross with *Solanum nigrum* or *Solanum dulcamara*. For oilseed rape and certain related species hybridisation is only possible with special techniques and extremely unlikely in natural conditions. The resulting hybrids do not persist in the environment.
Quantifying changes in establishment ability and competitiveness of genetically modified plants (BAP-0489/0490/0491/0492)(1989-1990) Method development project for the quantitative analysis of establishment ability, competitiveness and reproduction. The study subject was tobacco modified with a 35S:GUS construct.
Agronomic, environmental and genetic assessment of transgenic crop plants (BAP-0360/0418/0473) Method development project to perform and monitor small-scale field trials. Also, pollen flow studies were executed.
FP2 - BRIDGE Specific research and technological development programme (EEC) in the field of biotechnology (1987-1991)
Analysis of gene transfer between micro-organisms and plants (BIOT-CT91-0282)(1991-1994) The project examined whether genes may be transferred from plants to *Agrobacterium tumefaciens;* no evidence found
An experimental approach to investigate horizontal gene transfer between organisms (BIOT-CT91-0287)(1991-1993) The research model was *Cladosporium fulvum* and the host species tomato transformed with chimaeric retrotransposons with antibiotic resistance marker.
Safety assessment of the deliberate release of two model transgenic crop plants, oilseed rape and sugar beet (BIOT-CT91-0298)(1991-1993) Experiments on oilseed rape and subarbeet. The potential and likelihood for gene transfer to *Brassica campestris, B. adpressa, Sinapis arvensis, Raphanus raphanistrum* and *Beta maritima, B. atriplicifolia, B. macrocarpa* was studied and also the pollination ability to produce F1 hybrids under field and forced conditions, the reproductive potential of F1 hybrids and the competitiveness of hybrids in mixed populations.

[82] http://cordis.europa.eu/projects/home_en.html

Outcrossing was confirmed for *B. campestris*; for *B. adpressa, Sinapis arvensis, Raphanus raphanistrum* gene transfer was possible under specialised conditions. Hybridisation of sugarbeet is possible for *B. atriplicifolia* and *B. macrocarpa*, but less frequent as with *B. maritima*. Fitness of GMP not significantly different from the non-modified plants. Outcrossing confined to the immediate vicinity of the transgenic field.

FP3
(1990-1994)

Virus resistance in transgenic crop plants; influence of transport protein gene on viral host range, symptom expression on resistance of transgenic plants (MA2B910029)(1994-1998)

Basic research on viral infection about movement proteins for cell-to-cell transport.

Development of new methods for safety evaluation of transgenic food crops (AIR3-CT94-2311)(1995-1998)

On food safety.

FP4
(1994-1998)

Environmental impact of transgenic plants on beneficial insects (BIO4-CT96-0365)(1996-1999)

the studied NTOs are pollinating insects and insect parasitoids and the effects from proteinase inhibitors. Plant exposure and purified recombinant proteins are tested in artificial diets. More specifically honey bee on oilseed rape and potato and a parasitoid wasp. No acute toxicity was observed.

Risk assessment with genetically engineered woody plants expressing virus coat protein gene (BIO4-CT-0773)(1996-1999)

Study subjects: *Vitis* sp. and *Prunus* (also *Nicotiana* sp.). Natural vectors are used (aphids and nematodes) to study transencapsidation in greenhouse and field experiments.

Assessment of risks induced by virus-derived transgenic products in plants, using luteoviruses carrying the green fluorescent protein as a visible reporter (BIO4-CT98-0374)(1998-2000)

Basic research on BWYV (*beet western yellow virus*) and PLRV (*potato leaf roll virus*) with GFP as a reporter
Tests were developed to detect RNA recombinations, transencapsidations, synergism.

The mechanisms and control of genetic recombination in plants (BIO4-CT97-2028)(1997-2000)[83]

Basic research to study illegimate and homologous recombination to finally make gene targeting possible and to avoid silencing, multiple and scrambled copies and instability.

Control of gene expression and silencing in transgenic plants (BIO4-CT96-0253)(1996-2000)[84]

Basic research to understand mechanisms that regulate gene silencing.

Safety assessment of the release of transgenic crops: spread of herbicide resistance genes from wheat and foxtail millet to weedy species (IC18-CT98-0391)(1999-2002)

Hand-crossing, embryo-rescue, spontaneous interspecific hybridisations were studied to determine fitness and natural reproduction of the hybrids.

FP5
(1998-2002)

BT-BIONOTA
Effects and mechanisms of BT transgenes on biodiversity of non-target insects: pollinators, herbivores and their natural enemies (QLK3-2000-00547)(2000-2003)

4 toxin/crop combinations (maize expressing Cry1Ab, eggplant expressing Cry3B, potato expressing Cry1Ab, canola expressing Cry1Ac) were studied in laboratory feeding, greenhouse and field cage experiments. The mode of action in beneficial insects was studied using cDNA micro-arrays for changes in gene expression patterns. The insects were Green lacewing (predator), ladybirds, spiders, bumblebees, parasitoids.
Results: no harm on parasitoids, pollinators, predators, NTAs (field trials); effects on some predator species directly fed with Cry1Ab or Cry3Bb were seen.

POTATOCONTROL
Impact of three selected biotechnological strategies for potato pathogen control on the indigenous

[83] http://www.epsoweb.org/catalog/EU/science/projects/2028.htm (disfunctional)
[84] http://www.biology.leeds.ac.uk/centres/liba/eu_web/index.html (disfunctional)

soil microbiota (QLK3-2000-01598)(2001-2003)

One of the strategies was transgenic pathogen resistant potato (phage T4 lysozyme, lytic enzymes cecropin and/or attacin). The impact on microbiota (soil microbes) and genetic diversity of potato pathogens was investigated in greenhouse and field (Netherlands and Spain) experiments.
Results: no changes, site and growth phase posed stronger effects on the microbial community structure than the strategies used.

VRTP IMPACT

Virus-resistant transgenic plants: ecological impact of gene flow (QLK3-2000-00361)(2001-2004)[85]

Gene flow from plant to virus via recombination and plant to plant via sexual out-crossing was studied in laboratory and field studies. The study subjects were cucumoviruses and potyviruses; sugar beet and oilseed rape. Fitness advantage suggested.

EURICE

European Rice: Transgenes for crop protection against fungal diseases (QLK5-CT1999-01484)(2000-2003)

the project was to improve and evaluate genes acting as anti-fungal tools during fungal attack (*Magnaporthe grisea*) (inducible, tissue-specific promoters) the *afp* gene, isolated from the fungus *Aspergillus giganteus*, the insect antifungal gene *CecA* encoding for the antimicrobial protein Cecropin A, and a plant gene, the maize *b32* gene encoding for a RIP (Ribosome Inactivating Protein),
a.o. gene flow (*bar* gene)

NONEMA

Making plants resistant to plant parasitic nematodes: No access – No feeding (QLK5-CT-1999-1501 and QLK5-2001-02855)(2000-2004)

Very specific gene silencing prevents non-target effects.

Evaluating new traits for potato in the Central Andes with an appropriate poverty focus. (ICA4-2000-30019)(2000-2003)

The project studied the environmental effects of cystatins on non-target organisms, Evaluate the risks of nematode-resistant potato clones to non-target animals and soil micro-organisms in the central Andes, Also, gene flow from potato to wild relatives via pollen. Results: No change in the profile of rhizosphere bacteria; hand-crossing resulted in hybrids with all six wild species; gene flow occurred from a cultivated potato to 3 wild relatives in the field.

COTRAN

Assessment of the environmental and agronomic appropriateness of Bt transgenic cotton in small producer IPM systems in China (ICA4-CT-2000-10197)(2001-2005)

Bt management.
the aim was to improve the understanding of the impact of Bt cottons on other parts of the agro-ecosystem. There were no significant effects on non-target lepidoptera, whiteflies, cotton aphids, or springtail decomposers in the soil. Cotton bollworm, leafworms and cotton aphids could concentrate Bt toxin in their tissues while feeding and pass this on to predators. However, no evidence was found for significant disruption of the generalist predator complex in Bt cotton.

ECOSAFE

Biosafety research directed at more sustainable food production (QLK3-2000-01759)(2001-2003)

Effects on soil micro-organisms, GM inoculants
ecological consequences of the use of novel biotechnology products, based on GM plants and microbial inoculants, to meet the goals of sustainability in European agriculture. base-line effects of inoculation with wild-type *Azospirillum* from field trials and contained trials of GM inoculants. any impact of inoculants on bacterial diversity was less than variations due to natural soil heterogeneity/plant-to-plant variability, seasonal effects and plant age.
cell-cell communication: Produce transgenic plants which can produce bacterial signals. genetically modified plants did not have a negative ecological impact.

CIMES

Ciliates as monitors for environmental safety of GMO (QLK3-2002-02151)(2002-2005)

[85] http://www-biocel.versailles.inra.fr (disfunctional)

Horizontal (or lateral) gene transfer in eukaryotes. ciliates (unicellular eukaryotic microorganisms) would be ideal monitors to detect potential lateral gene transfer in the intestinal tracts of animals. Bt176 maize fed cows and pulverized leaves *in vitro* studies with ciliates. And GFP (plasmid).
The analysis of rumen ciliates challenged with Bt176 maize *in vitro* for two years, and of *Nyctotherus ovalis* (bacteriovorous ciliate) which was exposed to Bt176 for three years, failed to provide any evidence for the uptake of Bt176-related genes.

ANGEL
Analysis of gene flow from crop to wild forms in lettuce and chicory and its population-ecological consequences in the context of GM-crop biosafety (QLK3-CT-2001-01657)(2001-2005)

Non-GM chicory (obligatory outcrosser) and lettuce (selfing species) were the studied crops. Using microsatellites and AFLP markers and colour; fitness of hybrids (field and greenhouse) was examined. Chicory hybrids are as fit as wild type, with a good survival of hybrid lettuce.

TRANSBAC
Gene flow from transgenic plants: evaluation and biotechnology (QLK3-2001-02242)(2002-2005)

Gene transfer from transplastomic plants (lindane resistance) to bacteria (*Ralstonia solanacearum* & *Acinetobacter* sp.) was studied. Results: transfer from decaying plants was confirmed when sequence homology is available.

ProBenBT
Protecting the benefits of Bt-toxins from insect resistance development by monitoring and management (QLK3-CT-2002-01969)(2002-2006)

The baseline susceptibility in ECB and MCB to Bt-toxin (Cry1Ab toxin) before the cultivation of Bt-maize is measured. Development of genetic tools for detecting Bt resistance genes.

TRANSVIR
Environmental impact assessment of transgenic grapevines and plums on the diversity and dynamics of virus populations (QLK3-2002-02140)(2003-2006)

Evaluation of the variability of virus populations; monitor the emergence of recombinant virus species; both in GM and non-GM plants, field experiments
Grapevines expressing the CP gene of *Grapevine fanleaf virus* (GFLV), *Grapevine virus A* (GVA) or *Grapevine virus B* (GVB), and transgenic plums expressing the CP gene of *Plum pox virus* (PPV) were the study subjects.
Results: no effect of GM on diversity or recombination

ECOGEN
Soil ecological and economic evaluation of genetically modified crops (QLK5-CT-2002-01666)(2002-2006)

A wide range of taxonomic groups of soil organisms covering *Acari*, *Oligochaeta*, *Collembola*, *Protozoa*, *Nematoda* and *Mollusca* were investigated for direct sensitivity to Bt-toxin both in a pure chemical form and as part of the plant biomass of maize in first-tier laboratory screening tests. No effect.
Greenhouse experiments: The largest effects observed were from the soil type and plant growth stage.
Field experiments: no differences

FP6
(2002-2006)

CO-EXTRA
Results and perspectives on the coexistence and traceability of GM and non-GM supply chains[86] (007158)(2005-2009)

The study provided practical tools and methods for implementing co-existence (containment) and to enable traceability in a cost-effective manner.

SIGMEA
Sustainable Introduction of GMOs into European agriculture[87] (FP6-2002-SSP1-501986) (2004-2007)

Collected European gene flow data of maize, rapeseed, sugar beet, rice, wheat; designed

[86] GM and non-GM supply chains: their CO-EXistence and TRAceability'. Report summary http://cordis.europa.eu/result/rcn/53012_en.html
[87] http://www6.inra.fr/sigmea

predictive models of gene flow at the landscape level; Co-existence
environmental impacts of GM crops in EU: Bt-maize: none; HT oilseed rape: depletion of weed flora and dependent invertebrates; HT volunteers; beet: depletion of weed flora and dependent invertebrates

FP7
2007-2013

AMIGA
Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems[88] (KBBE.)(2011-2015)

Aims at producing scientific data related to the possible environmental and economic impacts of cultivation of GMPs that are relevant to European environments.
The project aims at

- providing baseline data on biodiversity in agro-ecosystems in the EU,
- translating regional protection goals in measurable assessment endpoints, (WP2)
- defining lists of suitable bioindicators for various European regions,
- improving knowledge on potential long term environmental effects of genetically modified plants (GMPs),
- testing the efficacy of the EFSA Guidance Document (GD) for the Environmental Risk Assessment (ERA) of GMPs,
- exploring new strategies for post-market monitoring,
- estimating the compatibility of GMPs with the Integrated Pest Management (IPM) principles implemented in the EU,
- providing a systematic analysis of economic aspects of GMPs cultivation in the EU, and
- setting a training and communication plan addressing public concerns about GMPs.

DARE
(COST Action TD0803): "Detecting Evolutionary Hot Spots of Antibiotic Resistance in Europe"[89] (2009-2013)

The main was to identify and characterize environmental hot spots for antimicrobial resistance (*e.g.* wastewater treatment plants and animal production), emergence and spreading of antibiotics and antibiotic resistance patterns, aiming at the development of measures to control antibiotic resistance evolution.

FEAR
(COST Action project) "Prevalence of aminoglycoside phosphotransferase genes (*npt*II/*npt*III) in natural habitats - Baseline acquisition for an improved risk assessment of ARM genes from GMOs"[90] (2010-2012)

One of the objectives is the acquisition of the baseline prevalence of *nptII* and *nptIII* in the microbial gene pool of representative naturally occurring bacterial populations

GRACE
GMO Risk Assessment and Communication of Evidence[91] (KBBE. 2012.3.5-04)(2012-2015)

Evidence Synthesis of GMO Impact Research, Questions:

- Systematic Review "Does the growing of Bt maize change populations or ecological functions of non-target animals compared to the growing of conventional non-GM maize?
- Systematic Review "Environmental effects of the cultivation of GM herbicide tolerant crops"
- Systematic Review "Are population abundances of soil invertebrates changed by Bt crops compared with conventional crops?"
- Systematic Review: "Target Insect Resistance Development and Resistance Management of Bt-Crops – Review Question: "Are data on base line susceptibility of different lepidopteran/coleopteran maize pests to Bt-proteins available?"
- Evidence Map: "Target Insect Resistance Development and Resistance Management of Bt-Crops – Review Question: "Is the inheritance of resistance alleles fully recessive in populations of lepidopteran/coleopteran maize pest?"
- Systematic Review "Effects of Bt crops on soil microorganisms" (NTO review question

[88] http://www.amigaproject.eu/
[89] http://www.cost-dare.eu/
[90] https://forschung.boku.ac.at/fis/suchen.projekt_uebersicht?sprache_in=en&menue_id_in=300&id_in=8268
[91] http://www.grace-fp7.eu/

3). Review Question: “Are soil microbial endpoints changed by Bt crops compared with conventional crops?”

Testing various types of animal feeding trials and alternative *in vitro* methods for health risk assessments of GM food and feed.

Results will appear on the GRACE website and in the CADIMA (Central Access Database for Impact Assessment of Crop Genetic Improvement Technologies) database[92].

Kohl C., Frampton G., Sweet J., Spök A., Haddaway N.R., Wilhelm R., Unger S., Schiemann J., 2015, Can systematic reviews inform GMO risk assessment and risk management? Frontiers in Bioengineering and Biotechnology 3, 113.

G-TwYST

Genetically modified plants Two Year Safety Testing,[93] (KBBE.2013.3.5-03)(2014-2018)

On food/feed safety testing

PreSto GMO ERA-Net[94]

Preparatory steps towards a GMO research ERA-Net (KBBE.)(2013-2015)

This project is focused on identifying gaps in existing knowledge regarding risk–benefit assessment and associated governance practice of GMOs in order to inform future research work, in particular regarding the European Research Area Network (ERA-NET) on GM research. It lays the groundwork for transnational research on health, environmental and techno-economic impacts of GMOs. The project engages stakeholders throughout all stages of the project to ensure that future research in this area will also be highly relevant and meaningful from a broader societal perspective.’

The project will create a sound basis for a robust ERA-Net proposal, by:

- Identifying gaps in the existing knowledge relevant for risk-benefit considerations of current and future GMO applications.
- Significantly improving the alignment of ongoing and future research programmes of the individual Member States, in order to avoid duplication of work, to leverage complementarities, and to enhance cooperation between scientists from all over Europe to create an internationally recognizable critical mass in terms of expertise and capacities.
- Promoting the accessibility of existing scientific information to interested stakeholders, regulators and end-users.
- Supporting an open communication and dialogue on all societally relevant issues associated with GMOs that takes into account both benefits and risks. This will allow making an informed choice about whether and how biotechnologies can be used to deliver solutions to the current and future challenges in agriculture and other areas.

Results will be available in CADIMA.

GMSAFOOD[95]

Biomarkers for post market monitoring of short and long-term effects of genetically modified organisms (GMOs) on animal and human health (KBBE-2007-2-5-01)(2008-2012)

On food/feed

Post-market monitoring to further assess possible nutritional and health effects of authorized GM foods.

[92] http://www.cadima.info/
[93] http://www.g-twyst.eu/
[94] http://www.presto-gmo-era-net.eu/
[95] http://cordis.europa.eu/project/rcn/87817_en.html

Table 4 ERGO projects, theses and publications

(as provided by the final ERGO report[96])

ERGO projects
Development of an ecological method to evaluate the effects of GM crucifer crops, altered in direct and indirect plant resistance traits, on non-target organisms in relation to baseline information
Effects of genetically modified crops on beneficial soil fungi
Quantifying introgression risks of transgenes with hazard rates, using carrot as a model species
Introgression of crop (trans-)genes into wild relatives: hybrid fitness, background selection and hitchhiking in *Lactuca serriola*; Tools for assessing the likelihood of the establishment of transgenes in wild relatives
Baseline establishment and protocol development to assess the effects of genetically modified crops on the structure and functioning of soil microbial communities
GM Crop Impact Assessment on Soil Ecosystems by DNA Barcode-based Monitoring of Nematode Communities (ERGONema)
Developing baselines and protocols for evaluating the direct and indirect effects of GMP's on the above-ground insect community using GM potatoes as a case study
The baseline of soil functioning across a representative range of Dutch soils
Design of a decision matrix to assess GM crop impact on the detrital food web
Whole-cell biosensors to monitor and assess the effects of transgenic crops on soil health
Potential ecosystem effects of future GM crop introductions through establishment of crop/wild hybrids or feral populations
Potential ecosystem effects of future GM crop introductions through establishment of crop/wild hybrids or feral populations
A decision-support system for plant traits constituting ecological risks
What elements are, ERA-wise, key to the 'description of new generation gmo crops, what traits/characteristics require asking further details on ecological behaviour?
ERGO theses
Kos M.,2012, Multitrophic effects of plant resistance: from basic ecology to application in transgenic crops
Houshyani, B., 2012, Application of omics technologies for environmental risk assessment of genetically modified plants; Arabidopsis and modified defense mechanisms as a model study
Kabouw P., 2012, Consequences of intra-specific metabolic diversity in plants for soil organisms: a baseline approach for evaluating ecological effects of genetic modifications,
Verbruggen E., 2012, Agriculture-induced changes in mycorrhizal fungal assemblages; Implications for ecological risk assessment of transgenic crops
Hillekens R., in preparation, Working title: Tracking basidiomycete communities in soil and potential impacts of GM crops on decomposing fungi.
Uwimana B., 2011, A genetic analysis of the introgression process from cultivated lettuce (*Lactuca sativa* L.) to wild prickly lettuce (*Lactuca serriola* L.)
Hartman Y., 2012, Genomic regions under selection in crop-wild hybrids of Lettuce; implications for crop breeding and environmental risk assessment
Inceoğlu Ö., 2011, Normal operating range of the microbial community under potato
Hannula E., 2012, Assessment of the effects of genetically modified potatoes on structure and functioning of soil fungal communities
Brolsma K., 2014, Crop-impact assessment on microbial mediated processes in agricultural soils
Vervoort J., 2013, Covering ground: Insights into soil ecology by molecular monitoring of nematode assemblages
Pereira e Silva M.C., 2013, The normal operating range of soil functioning: understanding the natural fluctuations of nitrogen cycling communities

[96] http://www.nwo.nl/actueel/nieuws/2014/alw/afsluitende-publicatie-programma-ecologie-rond-genetisch-gemodificeerde-organismen-ergo.html

Szturc-Koetsier, A., 2013, The normal operating range of carbon degradation in Dutch agricultural soils

van Ommen Kloeke E., 2013, Living in a broccoli world; design of a decision matrix for assessing the impact of novel (GM) crops on the soil ecosystem.

ERGO publications

Benyamin Houshyani, Patrick Kabouw, Dorota Muth, Ric C. H. de Vos, Raoul J. Bino and Harro J. Bouwmeester, 2011. Characterization of the natural variation in *Arabidopsis thaliana metabolome* by the analysis of metabolic distance. Metabolomics, in press DOI: 10.1007/s11306-011-0375-3

Benyamin Houshyani, Maryam Assareh, Antoni Busquets, Albert Ferrer, Harro J. Bouwmeester, Iris Kappers. Three-step pathway engineering results in more incidence rate and higher emission of nerolidol and improved attraction of *Diadegma semiclausum*. Submitted to Metabolic Engineering

Houshyani B, van der Krol SAR, Bino RJ, Bouwmeester HJ. Assessment of transcriptome perturbations in Arabidopsis lines with genetically engineered indirect insect defence. In preparation.

Houshyani B, Kos M, Ararsa B, van Loon JJA, Dicke M, Beekwilder J, Bouwmeester HJ. Overexpression of HAG1/MYB28 in metabolically diverged Arabidopsis accessions: Effect on glucosinolates, gene expression profiles and performance of specialist and generalist herbivores. In preparation.

Houshyani B, Kabouw P, Bouwmeester HJ. 2012 Whole-metabolome difference assessment of GM Arabidopsis lines with three novel insect defence traits using natural metabolome variation as a reference. In preparation.

Benyamin Houshyani, Patrick Kabouw, Ric de Vos & Harro Bouwmeester. Metabolic distance a new feature in metabolomics. Metabolomics. In press.

Patrick Kabouw, Maarten Nab & Nicole M. van Dam 2010. Activated carbon addition affects substrate pH and qermination of six plant species. Soil Biology & Biochemistry 42, 1165-1167.

Patrick Kabouw, Arjen Biere, Wim H. van der Putten & Nicole M. van Dam 2010. Intra-specific Differences in Root and Shoot Glucosinolate Profiles among White Cabbage (*Brassica oleracea* var. *capitata* Cultivars. Journal of Agricultural and Food Chemistry 58 (1 (411-417.

Patrick Kabouw, Wim H. van der Putten, Nicole M. van Dam & Arjen Biere 2010. Effects of intraspecific variation in white cabbage (*Brassica oleracea* var. *capitata* on soil organisms. Plant and Soil 336 (509-518.

Kabouw P., Kos M., Kleine S., Vockenhuber E.A., van Loon J.J.A., van der Putten W.H., van Dam N.M., Biere A. 2011. Effects of soil organisms on aboveground multitrophic interactions are consistent between plant genotypes mediating the interaction. Entomologia Experimentalis et Applicata: Vol. 139, pp 197-206.

Patrick Kabouw, Nicole van Dam, Wim van der Putten & Arjen Biere 2012. How genetic modification of roots affects rhizosphere processes and plant performance. Journal of Experimental Botany 63: 3475-3483.

Kos M., van Loon J.J.A., Dicke M., Vet L.E.M. 2009. Transgenic plants as vital components of integrated pest management. Trends in Biotechnology: Vol. 27, pp 621-627.

Kos M., Broekgaarden C., Kabouw P., Oude Lenferink K., Poelman E.H., Vet L.E.M., Dicke M., van Loon J.J.A. 2011. Relative importance of plant-mediated bottom-up and top-down farces on herbivore abundance on *Brassica oleracea*. Functional Ecology: Vol. 25, pp 1113-1124.

Kos M., Kabouw P., Noordam R., Hendriks K., Vet L.E.M., van Loon J.J.A., Dicke M. 2011. Prey-mediated effecs of glucosinolates on aphid predators. Ecological Entomology: Vol. 36, pp 377-388.

Martine Kos, Benyamin Houshyani, Buddhi B. Achhami, Rafal Wietsma, Rieta Gols, Berhane T. Weldegergis, Patrick Kabouw, Harro J. Bouwmeester, Louise E. M. Vet, Marcel Dicke and Joop J. A. van Loon, 2012. Herbivore-mediated effects of glucosinolates on different natural enemies of a specialist aphid. J Chem Ecol, in press

Kos M, Houshyani B, Wietsma R, Kabouw P, Vet LEM, van Loon JJA, Dicke M. 2012 Effects of glucosinolates on a generalist and specialist leaf-chewing herbivore and an associated parasitoid. Phytochemistry. In press.

Kos M, Houshyani B, Overeem AJ, Bouwmeester HJ, Weldegergis BT, van Loon JJA, Dicke D,

Vet LEM. 2012 Genetic engineering of plant volatile terpenoids: effects on a herbivore, a predator and a parasitoid. Pest Management Science. Submitted.

Gamper, H.A. and Heijden van der, M. and Kowalchuk, G.A. 2009 Molecular trait indicators: Moving beyond phylogeny in arbuscular mycorrhizal ecology. New Phytologist, 185, 67-82. ISSN 0028-646X;

García-Villaraco Velascoa A, Kowalchuk GA, Gutierrez Ma˜neroa FJ, Ramosa B, Yergeau E, Lucas García JA 2013. Increased microbial activity and nitrogen mineralization coupled to changes in microbial community structure in the rhizosphere of Bt corn. Applied Soil Ecology 68: 46– 56.81

Kowalchuk GA 2012 Bad news for soil carbon sequestration? Science, 337:1019-1050.

Kuramae EE, Gamper HA, Yergeau E, Piceno YM, Brodie EL, DeSantis TZ, Andersen GL, van Veen JA, Kowalchuk GA 2010. Microbial secondary succession in a chronosequence of chalk grasslands. The ISME Journal, 4: 711-715.

Kuramae EE, Gamper HA, van Veen JA, Kowalchuk GA 2011. Soil and plant factors driving the community of soil-borne microorganisms across chronosequences of secondary succession of chalk grasslands with neutral pH. FEMS Microbiology Ecology. 77: 285–294.

Kuramae EE, Yergeau E, Wonga LC, Pijl AS, van Veen JA, Kowalchuk GA 2012. Soil characteristics more strongly influence soil microbial communities than land management. FEMS Microbiology Ecology. 79: 12-24.

Mello A, Miozzi L, Vizzini A, Napoli C, Kowalchuk GA, Bonfante P 2010. Bacterial and fungal communities associated to Tuber magnatum-productive niches. Plant Biosystems, 144: 323–332.

Kielak A, Pijl AS, van Veen JA, Kowalchuk GA 2008. Plant species identity and diversity lead to only minor changes in soil-borne microbial communities in a former arable field. FEMS Microbiology Ecology, 63: 372-382

Verbruggen E, Röling WFM, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MGA. 2010. Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytologist 186: 968-979.

Verbruggen E, Kiers ET. 2010. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. Evolutionary Applications 3: 547-560.

Verbruggen, E., Kiers, E.T., Bakelaar, P.N.C., Röling, W.F.M., van der Heijden, M.G.A. 2012. Soil communities from organically and conventionally managed fields provide different agro-ecosystem services. Plant and Soil 350: 43-55 (with commentary).

Verbruggen, E., van der Heijden, M.G.A., Weedon, J.T, Kowalchuk, G.A., & Röling W.F.M 2012. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. Molecular Ecology , 21: 2341-2353.

Verbruggen E, Hillekens R, Kuramae E, de Hollander M, Kiers E.T., Röling W.F.M., Kowalchuk G.A and van der Heijden M.G.A. 2012. Testing Potential Effects of Maize Expressing the Bacillus thuringiensis Cry1Ab Endotoxin (Bt Maize on Mycorrhizal Fungal Communities via DNA- and RNA-Based Pyrosequencing and Molecular Fingerprinting. Applied Environmental Microbiology 78: 7384-7392.

Ghosh A, P Haccou. 2010. Quantifying stochastic introgression processes with hazard rates. Theor. Pop. Biol. 77:171-180.82

Ghosh A, M Conceicao Serra, P Haccou. 2012. Quantifying time-inhomogeneous stochastic introgression processes with hazard rates. Theor. Pop. Biol. 81 253–263.

Ghosh A, P. Meirmans, P Haccou. Quantifying introgression risk with realistic population genetics. Proc. Roy. Soc. (accepted)

Grebenstein C, Choi YH, Rong J, de Jong TJ, Tamis WL: Metabolic fingerprinting reveals differences between shoots of wild and cultivated carrot (*Daucus carota L*. and suggests maternal inheritance or wild trait dominance in hybrids. Phytochemistry; 2011; 72(11-12:1341-7

Grebenstein C, Kos SP, de Jong TJ, Tamis WLM, de Snoo GR: Morphological markers for the detection of introgression from cultivated into wild carrot (*Daucus carota L*. reveal dominant domestication traits. Plant Biology; (in press)

Grebenstein C, a.o.: Demography and life history of wild carrot (*Daucus carota L*. populations in The Netherlands. (in preparation)

Grebenstein C, a.o. Respective fitness of Wild x Cultivar carrot Daucus carota L. hybrid and

backcross lines shows potential for introgression (preliminary title. (in preparation)

Rong J, Janson S, Umehara M, Ono M, Vrieling K. 2010. Historical and contemporary gene dispersal in wild carrot (Daucus carota ssp. carota populations. Annals of Botany 106: 285-296.

Rong J, Song ZP, de Jong TJ, Zhang XS, Sun SG, Xu X, Xia H, Liu B, Lu BR. 2010. Modelling pollen-mediated gene flow in rice: risk assessment and management of transgene escape. Plant Biotechnology Journal 8: 452-464.

Rong J, Xu S, Vrieling K. 2012. Dissimilarity of contemporary and historical gene flow in wild carrot metapopulation under strong human disturbance.

Rong J, Lammers Y, Schidlo NS, Ariyurek Y, de Jong TJ, Klinkhamer PGL, Vrieling K. 2012. New insights into crop domestication using high-throughput transcriptome sequencing of cultivars and wild populations. (submitted)

Ghosh, A., Meirmans, P.G., Haccou, P. Quantifying introgression risk with realistic population genetics. Submitted to Proceeding of the Royal Society B.

Hartman, Y., Hooftman, D.A.P., Uwimana, B, Wiel, C.C.M. van de, Smulders, M.J.M., Visser, R.G.F. & Tienderen, P.H. van (2012. Genomic regions in crop–wild hybrids of lettuce are affected differently in different environments: implications for crop breeding. *Evolutionary Applications*, in press.

Hartman, Y., Uwimana, B., Hooftman, D.A.P., Schranz, M.E., Wiel, C.C.M. van de, Smulders, M.J.M., Visser, R.G.F. & Tienderen, P.H. van. Genomic and environmental selection patterns in two distinct lettuce crop-wild hybrid crosses. Submitted to Evolutionary Applications.

Hartman, Y., Hooftman, D.A.P., Schranz, M.E., & Tienderen, P.H. van. QTL analysis reveals the domestication architecture of Crisphead lettuce. Submitted to Genetic Resources and Crop Evolution.

Hengstum, T. van, Hooftman, D.A.P., Nijs, H.C.M. den & Tienderen, P.H. van. 2012 Does insect netting affect the containment of airborne pollen from GM- plants in greenhouses? Aerobiologia, 28, 325-335,

Hengstum, T. van, Lachmuth, S., Oostermeijer, J.G.B., Meirmans, P.G. & Tienderen, P.H. van. 2012 Human-induced hybridization among congeneric endemic plants on Tenerife, Canary Islands. Plant Systematics and Evolution, 298, 603-618.

Hooftman, D.A.P., Nieuwenhuis, B.P.S., Posthuma, K.I., Oostermeijer, J.G.B. & Nijs, J.C.M. den, 2007 Introgression potential of downy mildew resistance from lettuce to Lactuca serriola and its relevance for plant fitness. Basic and Applied Ecology, 8, 135-146.

Hooftman, D.A.P., Jong, M.A. de, Oostermeijer, J.G.B. & Nijs, J.C.M. den, 2007 Modelling the long-term consequences of crop–wild relative hybridization: a case study using four generations of hybrids. Journal of Applied Ecology, 44, 1035-1045.

Hooftman, D.A.P., Oostermeijer, J.G.B., Marquard, E. & Nijs, J.C.M. den, 2008 Modelling the consequences of crop–wild relative gene flow: A sensitivity analysis of the effects of outcrossing rates and hybrid vigour breakdown in *Lactuca*. Journal of Applied Ecology, 45(4, 1094-1103.

Hooftman, D.A.P., Hartman, Y., Oostermeijer, J.G.B. & Nijs, J.C.M. den. 2009 Existence of vigorous lineages of crop-wild hybrids in Lettuce under field conditions. Environmental Biosafety Research, 8(04, 203-217.

Hooftman, D.A.P., Flavell, A.J., Jansen, H., Nijs, H.C.M. den, Syed, N.H, Sorensen, A.P, Orozco-ter Wengel, P., Wiel, Clemens C. M. van de. 2011 Locus-dependent selection in crop-wild hybrids of lettuce under field conditions and its implication for GM crop development. Evolutionary Applications, 4, 648-659.

Meirmans, P.G., Bousquet, J. & Isabel, N. 2009 A metapopulation model for the introgression from Genetically Modified Plants into their wild relatives. Evolutionary Applications, 2, 160-171.

Meirmans, P.G., Lamothe, M., Gros-Louis, M.C., Khasa, D., Périnet, P., Bousquet, J. & Isabel, N. 2010 Complex patterns of hybridization between exotic and native North American poplar species. American Journal of Botany, 97(10, 1688-1697.

Meirmans, P.G. & Hedrick, P.W. 2011 Invited review: Assessing population structure: FST and related measures. Molecular Ecology Resources, 11(1), 5-18.

Meirmans, P.G., Goudet, J. & Gaggiotti, O.E. 2011 Ecology and life history affect different aspects of the population structure of 27 high-alpine plants. Molecular Ecology, 20, 3144-3155.

Meirmans, P.G. 2011 MARLIN, software to create, run, and analyse spatially realistic

simulations. Molecular Ecology Resources, 11(1), 146-150.

Meirmans, P.G. 2012 AMOVA-based clustering of population genetic data. Journal of Heredity, 10.1093/jhered/ess047.

Meirmans, P.G. 2012 The trouble with isolation by distance. Molecular Ecology, 21, 2839-2846.

Meirmans, S., Meirmans, P.G. & Kirkendall, L.R. 2012 The costs of sex: facing real-world complexities. Quarterly Review of Biology, 87(1, 19-40.

Meirmans, P.G., Tienderen, P.H. van. The effects of inheritance in tetraploids on genetic diversity and population divergence. Accepted (minor revisions by Heredity)

Neiman, M., Meirmans, S. & Meirmans, P.G. 2009 What can asexual lineage age distribution tell us about the maintenance of sex? Annals of the New York Academy of Sciences, 1168, 185-200.

Pelgas, B., Bousquet, J., Meirmans, P.G., Ritland, K. & Isabel, N. 2011 QTL mapping in white spruce: gene maps and genomic regions underlying adaptive traits across pedigrees, years and environments. BMC Genomics, 12, 145.

Rong, J., Xu, S., Meirmans, P.G., Vrieling, K. Dissimilarity of contemporary and historical gene flow in plant metapopulation under strong human disturbance: implications in risk assessment and management of transgene introgression. Submitted to Evolutionary Applications.

Thompson, S.L., Lamothe, M., Meirmans, P.G., Périnet, P. & Isabel, N. 2010 Repeated unidirectional introgression towards *Populus balsamifera* in contact zones of exotic and native poplars. Molecular Ecology, 19(1, 132-145.

Brigitte Uwimana, Luigi D'Andrea, François Felber, Danny A.P. Hooftman, Hans C.M. Den Nijs, Marinus J.M. Smulders, Richard G.F. Visser, Clemens C.M. van de Wiel. 2012 Molecular Ecology Volume 21, Issue 11, pages 2640–2654, June 2012.

A Bayesian analysis of gene flow from crops to their wild relatives: cultivated (*Lactuca sativa L*. and prickly lettuce (*L. serriola L*., and the recent expansion of *L. serriola* in Europe.

Uwimana, B, Smulders, M.J.M., Hooftman, D.A.P., Hartman, Y., Tienderen, P. van, Jansen, J., McHale, L.K., Michelmore, R.W., Visser, R.G.F. & Wiel, C.C.M. van de. 2012 Crop to wild introgression in lettuce: following the fate of crop genome segments in backcross populations. BMC Plant Biol, 12.

Uwimana, B., Smulders, M.J.M., Hooftman, D.A.P., Hartman, Y., van Tienderen, P.H., Jansen, J., McHale, .L.K., Michelmore, R.W., van de Wiel, C.C.M., Visser, R.G.F. Hybridization between crops and wild relatives: the contribution of cultivated lettuce to the vigour of crop-wild hybrids under drought, salinity and nutrient deficiency conditions. Theoretical and Applied Genetics, in press.

Van den Berg, P., Weissing, F.J., Hooftman, D.A.P. & Bijlsma, R. 2012 Modeling the risk of transgene spread and establishment to multiple target populations. Manuscript in preparation

Dias ACF, EF Hoogwout, M de Cassia Pereira e Silva, J.F. Salles, L S van Overbeek, J.D. van Elsas, 2012. Potato cultivar type affects the structure of ammonia oxidizer communities in field soil under potato beyond the rhizosphere. Soil Biol. Biochem 50: 85-95.

Dias ACF, ES Hannula, F. Dini-Andreote, F. Dini Andreote, JF Salles, J D van Elsas. 2012. Differential selective effects on rhizosphere bacteria exerted by conventional and genetically modified potato lines. New Phytologist (in review)

Emilia Hannula, S.E., W. de Boer & J.A. van Veen, 2010 In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. Soil Biology & Biochemistry 42: 2211-2223.

Hannula, S.E., H. T. S. Boschker, W. de Boer & J. A. van Veen, 2012 13C pulse-labeling assessment of the community structure of active fungi in the rhizosphere of a genetically starch-modified potato (Solanum tuberosum cultivar and its parental isoline, New Phytologist 194, 784-799.

Hannula S.E., de Boer W, & J.A.van Veen J. 2012 A 3-year study reveals that plant growth stage, season and field site affect soil fungal communities while cultivar and GM-trait have minor Effects. PloS One 7(4: e33819.

Hannula S.E.,W. de Boer, P.Baldrian, J.A. van Veen. 2012 Effects of genetically modified amylopectin-accumulating potato in decomposer processes and fungal diversity in litter and soil. (submitted)

Inceoğlu 0, Salles JF, van Overbeek L, van Elsas JD. 2010 Effects of plant genotype and growth

stage on the betaproteobacterial communities associated with different potato cultivars in two fields. Appl Environ Microbiol. 76: 3675-3684.

Dias ACF, EF Hoogwout, M de Cassia Pereira e Silva, J.F. Salles, L S van Overbeek, J.D. van Elsas, 2012. Potato cultivar type affects the structure of ammonia oxidizer communities in field soil under potato beyond the rhizosphere. Soil Biol. Biochem 50: 85-95.

Dias ACF, ES Hannula, F. Dini-Andreote, F. Dini Andreote, JF Salles, J D van Elsas. 2012. Differential selective effects on rhizosphere bacteria exerted by conventional and genetically modified potato lines. New Phytologist (in review)

Emilia Hannula, S.E., W. de Boer & J.A. van Veen, 2010 In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. Soil Biology & Biochemistry 42: 2211-2223.

Hannula, S.E., H. T. S. Boschker, W. de Boer & J. A. van Veen, 2012 13C pulse-labeling assessment of the community structure of active fungi in the rhizosphere of a genetically starch-modified potato (Solanum tuberosum cultivar and its parental isoline, New Phytologist 194, 784-799.

Hannula S.E., de Boer W, & J.A.van Veen J. 2012 A 3-year study reveals that plant growth stage, season and field site affect soil fungal communities while cultivar and GM-trait have minor Effects. PloS One 7(4: e33819.

Hannula S.E.,W. de Boer, P.Baldrian, J.A. van Veen. 2012 Effects of genetically modified amylopectin-accumulating potato in decomposer processes and fungal diversity in litter and soil. (submitted)

Inceoğlu 0, Salles JF, van Overbeek L, van Elsas JD. 2010 Effects of plant genotype and growth stage on the betaproteobacterial communities associated with different potato cultivars in two fields. Appl Environ Microbiol. 76: 3675-3684.

Inceoglu 0, Hoogwout EF, Hili P, van Elsas JO. 2010 Effect of DNA extraction method on the apparent microbial diversity of soil. Appl Environ Microbiol. 76: 3378-3382.

Inceoglu 0, W Abu Al-Soud, J. F. Salles, A.V. Semenov, J.D. van Elsas. 2011 4 analysis of bacterial communities in a potato field as determined by pyrosequencing. PLoS One 6: e23321.

Inceoglu 0, J. Falcao Salles, J.D. van Elsas. 2012 Soil and cultivar type shape the bacterial community in the potato rhizosphere. Microb. Ecol. 63: 460-470.

Inceoglu 0, JF Salles, AV Semenov, LS van Overbeek, JD van Elsas. 2012 The normal operating range of bacterial communities in soil used for potato cropping. Appl. Environ Microbiol (in review)

Inceoglu 0, JD van Elsas, JF Salles. 2012 Shifts in bacterial communities associated with the potato rhizosphere in response to aromatic sulfanate soil amendments. Applied Soil Ecology (in press)

Mulder C, Helder J, Vervoort MTW, Vonk JA. 2011 Trait-mediated diversification in nematode predator-prey systems. Ecology and Evolution 1: 386-391

Vervoort MTW, Vonk JA, Mooijman PJW, Van den Elsen SJJ, Van Megen HHB, Veenhuizen P, Landeweert R, Bakker J, Mulder C, Helder J. 2012 SSU ribosomal DNA-based monitoring of nematode assemblages reveals distinct seasonal fluctuations within evolutionary heterogeneous feeding guilds. PLoS ONE

Vonk JA, Mulder C (2013 Contrasting influence of soil nutrients and microbial community on differently sized basal consumers. Naturwissenschaften 100

Vervoort MTW, Vonk JA, Brolsma KM, Schütze W, De Goede RGM, Hoffland E, Bakker J, Mulder C, Hallmann J & Helder J, Release of isothiocyanates does not explain the effects of biofumigation with Indian mustard cultivars on nematode assemblages (submitted)

Andreas Plischke; Young Hae Choi; Paul M. Brakefield; Peter G.L. Klinkhamer; Maaike Bruinsma 2012. Metabolomic Plasticity in GM and Non-GM Potato Leaves in Response to Aphid Herbivory and Virus Infection. Journal of Agricultural and Food Chemistry 60 (6 1488-1493.

Andreas Plischke, Maaike Bruinsma, Peter G.L. Klinkhamer, Frans J.A. Jacobs. The use of standardized effect sizes in ecological risk assessment of GM plants. Submitted to Journal of Applied Ecology.

Nguyen Esmeralda López Lozano, Pereira e Silva MC, Poly F, Guillaumaud N; van Elsas JD and Salles JF. Seasonal variations in the abundance and activity of denitrifying communities across agricultural soils (In preparation)

Pereira e Silva MC, Semenov AV, van Elsas JD and Salles JF, 2011 Seasonal variations in diversity and abundance of diazotrophic communities across soils. FEMS Microbiology Ecology 77:57-68.

Pereira e Silva MC, Poly F, Guillarmaud N, van Elsas JD and Salles JF, 2012 Fluctuations in ammonia oxidizer communities are driven by soil structure and pH Frontiers in Terrestrial Microbiology 3:77

Pereira e Silva MC, Semenov AV, Heike Schmitt, van Elsas JD and Salles JF, 2012 The normal operating range of soil functioning: a plausible or faulty concept. (in press; Soil Biology and Biochemistry)

Pereira e Silva MC, Dias ACF, van Elsas JD and Salles JF. Spatial and temporal variation of archaeal, bacterial and fungal communities in agricultural soils. (under revision Plos One)

Pereira e Silva MC, Hai B, Poly F, Schloter M, van Elsas JD and Salles JF. Temporal dynamics of abundance and composition of N-fixing communities across agricultural soils. (in preparation)

Schmitt H., Szturc-Koetsier, A., Greve, G.D., Kuramae, E. Bacterial and fungal plant matter degradation for different soils and plant tissues (submitted or in preparation)

Semenov AV, Pereira e Silva MC, Szturc AE, Schmitt H, Salles JF and van Elsas JD. 2012 Impact of incorporated fresh 13C potato tissues on microbial community composition in soil. Soil Biology and Biochemistry 49:88-95.

Semenov AV, Pereira e Silva MC, Schmitt H, van Elsas JD and Salles JF. Quantitative assessment of soil functioning across a representative range of Dutch soils (In preparation)

Szturc-Koetsier, A., Greve, G.D., Schmitt, H. The normal operating range of the structure and function of bacterial carbon degradation in agricultural soils (submitted or in preparation)

Szturc-Koetsier, A., Greve, G.D., Schmitt, H. Structure and function of potato leaf degraders - the normal operating range (submitted or in preparation)

Szturc-Koetsier, A., Greve, G.D., Schmitt, H. Soil disturbances push carbon degradation out of its normal operating range (submitted or in preparation)

Szturc-Koetsier, A., Kuramae, E., Greve, G.D., Schmitt, H. Bacterial and fungal succession in potato leaf degradation (submitted or in preparation)

Castro-Ferreira, M. P.; Roelofs, D.; van Gestel, C. A. M.; Verweij, R. A.; Soares, A. M. V. M.; Amorim, M. J. B., Enchytraeus crypticus as model species in soil ecotoxicology. Chemosphere 2012, (87, 1222-1227.

Costa, D.; Mariën, J.; Janssens, T. K. S.; van Gestel, C. A. M.; Driessen, G.; Sousa, J. P.; van Straalen, N. M.; Roelofs, D., Influence of adaptive evolution of cadmium tolerance on neutral and functional genetic variation in *Orchesella cincta. Ecotoxicology* 2012, (21, 1-10.

De Boer, M. E.; Berg, S.; Timmermans, M. J. T. N.; Den Dunnen, J. T.; Van Straalen, N. M.; Ellers, J.; Roelofs, D., High throughput nano-liter RT-qPCR to classify soil contamination using a soil arthropod. Bmc Molecular Biology 2011, 12, 11.

De Boer, T. E.; Birlutiu, A.; Bochdanovits, Z.; Timmermans, M.; Dijkstra, T. M. H.; Van Straalen, N. M.; Ylstra, B.; Roelofs, D., Transcriptional plasticity of a soil arthropod across different ecological conditions. Molecular Ecology 2011, 20, 1144-1154.

De Boer, T. E.; Taş, N.; Braster, M.; E.J., T.; Röling, W. F.; Roelofs, D., The Influence of Long-Term Copper Contaminated Agricultural Soil at Different pH Levels on Microbial Communities and Springtail Transcriptional Regulation. Environmental Science and Technology 2012, 46, (1, 60-68.

Janssens, T.; Giesen, D.; Mariën, J.; van Straalen, N.; van Gestel, C.; Roelofs, D., Narcotic mechanisms of acute toxicity of chlorinated anilines in Folsomia candida (Collembola revealed by gene expression analysis. Environment international 2011, 37, 929-939.

Nota, B.; Vooijs, R.; van Straalen, N. M.; Roelofs, D., Expression of mtc in Folsomia candida indicative of metal pollution in soil. Environmental Pollution 2011, 159, 1343- 1347.

Roelofs, D.; de Boer, M.; Agamennone, V.; Bouchier, P.; Legler, J.; van Straalen, N., Functional environmental genomics of a municipal landfill soil. Frontiers in Genetics 2012, (3, 85.

Van Ommen Kloeke, A. E. E.; Douma, J. C.; Ordonez, J. C.; Reich, P. B.; van Bodegom, P. M., Global quantification of contrasting leaf life span strategies for deciduous and evergreen species in response to environmental conditions. Global Ecology and Biogeography 2012, 21, (2, 224-235.

Van Ommen Kloeke, A. E. E.; van Gestel, C. A. M.; Styrishave, B.; Hansen, M.; Ellers, J.; Roelofs, D., Molecular and life-history effects of a natural toxin on herbivorous and non-target soil

arthropods. Ecotoxicology 2012, 2078-2087.

Van Ommen Kloeke, A.; Jager, T.; van Gestel, C. A. M.; Ellers, J.; Pomeren, M.; Krommenhoek, T.; Styrishave, B.; Hansen, M.; Roelofs, D., Time-related survival effects of two gluconasturtn hydrolysis products on the terrestrial isopod *Porcellio scaber*. Chemosphere 2012, (89, 1084-1090.

Van Ommen Kloeke, A. E. E.; van Gestel, C. A. M.; Gong, P.; Ellers, J.; Roelofs, D., Toxic effects of a natural toxin on the life-history and gene expression of *Eisenia andrei*. BMC Genomics, in prep.

Van Straalen, N. M.; Janssens, T. K. S.; Roelofs, D., Micro-evolution of toxicant tolerance: from single genes to the genome's tangled bank. Ecotoxicology 2011, (20, 1-6.89

Van Straalen, N. M.; Roelofs, D., An introduction to ecological genomics (hardback, 2011)

Van Straalen, N.; Roelofs, D., Mining animal genomes to disclose physiological functions for adaptation to stress. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 2012, 163, S3.

Visser, B.; Roelofs, D.; Hahn, D. A.; Teal, P. E. A.; Mariën, J.; Ellers, J., Transcriptional Changes Associated with Lack of Lipid Synthesis in Parasitoids. Genome Biology and Evolution 2012, (8), 752-762.

Waagner, D.; Bayley, M.; Mariën, J.; Holmstrup, M.; Ellers, J.; Roelofs, D., Ecological and molecular consequences of prolonged drought and subsequent rehydration in *Folsomia candida* (Collembola. Journal of Insect Physiology 2011, 58, 130-137.

Diana L. Zuluaga, A. E. Elaine van Ommen Kloeke, Neil Graham, Ruud Verkerk, Wilfred F. M. Röling, Jacintha Ellers, Dick Roelofs and Mark G.M. Aarts. Biofumigation effects of a wild *Brassica oleracea* on beneficial soil invertebrates. To be submitted to New Phytologist.

Diana L. Zuluaga, Neil Graham, Annett Klinder, Carol Wagstaff, Ruud Verkerk and Mark G.M. Aarts. Overexpression of the MYB29 transcription factor increases the beneficial effects of *Brassica oleracea* plants for agriculture and human health. To be submitted to the Plant Journal.

Bakker PAHM, Doornbos RF, Zamioudis C, Berendsen RL, and Pieterse CMJ 2012 Induced systemic resistance and the rhizosphere microbiome. Plant Pathol J. In press.

Berendse RL, Pieterse CMJ, and Bakker PAHM 2012 The rhizosphere microbiome and plant health. Trends in Plant Science 17:478-486.

Chapelle E, Mendes R, Bakker PAHM and Raaijmakers JM 2012 Fungal invasion of the rhizosphere microbiome. In preparation

Doornbos RF, Van Loon LC, and Bakker PAHM (2012. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. Agronomy for Sustainable Development 32: 227-243.

Mendes R, Kruijt M, De Bruijn I, Dekkers E, Van der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM 2011 Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097-1100.

Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M (2010 Natural functions of lipopeptides from *Bacillus* and *Pseudomonas* species: more than surfactants and antibiotics. FEMS Microbiology Reviews 34:1037-1062.

Watrous J, Roach PJ, Alexandrov T, Heath BS, Yang JY, Kersten,R, van der Voort M, Pogliano K, Gross H, Raaijmakers JM, Moore BS, Laskin J, Bandeira N and Dorrestein PC. 2012 Mass spectral molecular networking of living microbial colonies. Proceedings National Academy of Sciences USA. E1743-E1752

T.J. de Jong & E. Hesse 2012 Selection against hybrids in mixed populations of Brassica rapa and B. napus: model and synthesis. *New Phytologist* 194: 1134-1142.

TJ de Jong & J Rong 2013 Crop-to-wild gene flow; does more sophisticated research provide better risk assessment? Environmental Science & Policy 27: 135-140.

T.J. de Jong, M. Tudela Isanta, E. Hesse (Submitted) Slug herbivory limits recruitment of Brassica accessions with low levels of aliphatic glucosinolates.

M. Moshgani M, Kolvoort E, de Jong TJ (submitted/in press; entry by project coordinator not completely clear about authorship and status) Comparison of the wild Brassica rapa and the crop B. napus: characters relevant for the persistent seedbank in the soil. Seed Science Research

T.J. de Jong, M. Moshgani, E. Kolvoort, E. Hesse. Glucosinolates protect Brassica seedlings

against slug herbivory. (in preparation)

Ellstrand, N.C., Meirmans, P., Rong J., Bartsch, D., Ghosh A., de Jong, T.J., Haccou P, Lu, B., Snow, Stewart, C.N., Strasburg, J., Tienderen, P.H., Vrieling L, Hooftman, D.A.P. 2013 Introgression of crop alleles into wild and weedy populations. Annual Reviews of Ecology, Evolution & Systematics. Volume 44, December 2013 (in press)

Heijning, P.T.M., Meirmans, P.G., W. Bouten & Tienderen, P.H. Wild relatives and the potential impact of increased introgression of transgenes (in preparation)

Hengstum, T. van, Oostermeijer, J.G.B. & Tienderen, P.H. van. The effect of plant invasions on local invertebrate assemblages and the role of apparent competition (in preparation)

Hengstum, T. van, Oostermeijer, J.G.B., Hooftman, D.A.P. & Tienderen, P.H. van. The effect of invasive plant species on invertebrate communities: a meta-analysis. (in preparation)

Hesse, K. van der Burg, A. F. D. Kamp, N. M. van Dam & T. J. de Jong. Variation in herbivore-induced responses in *Brassica rapa* and *B. napus.* (in preparation)

Hesse, A.F.D. Kamp, T.J. de Jong. The effect of glucosinolates on pre-dispersal seed predation in *Brassica rapa* and *B. napus*. (in preparation)

Hesse, A. F. D. Kamp & T. J. de Jong. Population persistence of *Brassica*: the effect of cultivation on life-history traits. (in preparation)

Hesse, A. F. D. Kamp & T. J. de Jong. Which plant characters influence Brassica population persistence? (in preparation)

Hesse, A. F. D. Kamp & T. J. de Jong. Temporal and spatial variation affect population persistence. (in preparation)

Annex 4 Data requirements

Table 1 Relationship between areas of concern in ERAs and data requirements for higher plants

EU (plants) Areas of concern according to DIR 2001/18/EC	Information according to Annex III B	Data
1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.	B. Information relating to (a) the recipient or (b) (where appropriate) parental plants C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified. 3. Information on the expression of the insert: 4. Information on how the genetically modified plant differs from the recipient plant in: a. mode(s) and/or rate of reproduction; b. dissemination; c. survivability. 5. Genetic stability of the insert and phenotypic stability of the GMHP 13. Information about previous releases of the genetically modified plant, if applicable.	- Biology document: survival, dispersal - Molecular characterization: genetic stability, expression levels - Laboratory experiments: viability, germination, sprouting, rooting and establishment (seed persistence) - Field trials: agronomic performance, phenotypic stability, competing ability
2. Any selective advantage or disadvantage conferred to the GMHP.	B. Information relating to (a) the recipient or (b) (where appropriate) parental plants C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified. 2. Information on the sequences actually inserted/deleted 3. Information on the expression of the insert: 13. Information about previous releases of the genetically modified plant, if applicable.	- Biology document: survival, dispersal - Molecular characterization: analysis of the vector and insert, expression levels
3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.	B. Information relating to (a) the recipient or (b) (where appropriate) parental plants C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified. 3. Information on the expression of the insert: 6. Any change to the ability of the GMHP to transfer genetic	- Biology document: reproductive biology - Molecular characterization: expression levels

		material to other organisms. 13. Information about previous releases of the genetically modified plant, if applicable.	
	- Plant to micro-organisms gene transfer		- Molecular characterization: prokaryotic promoters, homologous sequences, mobile elements, the presence of antibiotic resistance genes. - The presence of recipient micro-organisms, persistence of GM plant material (soil, digestive tract)
4.	Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).	C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified. 3. Information on the expression of the insert: 9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable). 13. Information about previous releases of the genetically modified plant, if applicable.	- Molecular characterization: expression levels - Literature study: • biology, life cycle, ecology and/or behaviour of the target organism; • distribution of the target organism; • host range of the target organism; • population genetics; • frequency of resistance (individuals or alleles); • mode of action of the GM plant product; • baseline susceptibility of the target organism to the transgenic products.
5.	Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.	C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified. 3. Information on the expression of the insert: 9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable). 10. Potential changes in the interactions of the GMHP with non-target organisms resulting from the genetic modification. 13. Information about previous releases of the genetically modified plant, if applicable.	- Molecular characterization: expression levels - Literature study: exposure studies with the same newly expressed protein - Laboratory experiments: exposure of selected NTOs to the newly expressed protein - Semi-field tests (if necessary) - Field studies (if necessary) - Compositional analysis
6.	Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions	B. Information relating to (a) the recipient or (b) (where appropriate) parental plants C. Characteristics of the genetically modified plant	- Biology document: key food and feed nutrients, anti-nutrients and toxicants - Molecular characterization: expression

	of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).	1. Description of the trait(s) and characteristics which have been introduced or modified. 3. Information on the expression of the insert: 7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification. 13. Information about previous releases of the genetically modified plant, if applicable.	levels - Desk study: allergenicity, toxicity - Compositional analysis - Animal feeding studies
7.	Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.	B. Information relating to (a) the recipient or (b) (where appropriate) parental plants C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified. 3. Information on the expression of the insert: 7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification. 8. Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the GMHP is intended to be used in animal feedstuffs 13. Information about previous releases of the genetically modified plant, if applicable.	- Biology document: key food and feed nutrients, anti-nutrients and toxicants - Molecular characterization: expression levels - Desk study: allergenicity, toxicity - Compositional analysis - Animal feeding studies
8.	Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).	C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified. 3. Information on the expression of the insert: 11. Potential interactions with the abiotic environment. 13. Information about previous releases of the genetically modified plant, if applicable.	- Molecular characterization: expression levels - Desk study: comparing the cultivation practices - Laboratory experiments (if necessary) - Compositional analysis: lignin fraction, C/N ratio
9.	Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.	B. Information relating to (a) the recipient or (b) (where appropriate) parental plants C. Characteristics of the genetically modified plant 1. Description of the trait(s) and characteristics which have been introduced or modified.	- Biology document: cultivation practices - Desk study: comparing the cultivation practices, scenario analysis - Desk study: geographical areas

Table 2 Relationship between areas of concern in ERAs and data requirements for organisms other than higher plants

EU (non-plants) Areas of concern according to DIR 2001/18/EC	Information according to Annex III A	Data
1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).	**THE GMO** A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s) B. Characteristics of the vector C. Characteristics of the modified organism 1. Information relating to the genetic modification: 2. Information on the final GMO (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed; (b) structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism; (c) stability of the organism in terms of genetic traits; (e) activity of the expressed protein(s); (h) history of previous releases or uses of the GMO; **CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT** A. Information on the release B. Information on the environment (both on the site and in the wider environment): **THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT** A. Characteristics affecting survival, multiplication and dissemination B. Interactions with the environment 1. predicted habitat of the GMOs, 2. studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses, 5. measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability, 6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation,	- Literature study: on donor, the recipient or parental organism - Data on vector/GMO production, purification and verification (qPCR assay, bio-assay) - Molecular characterization: genetic stability, **characterization of the newly expressed protein** - Treatment data: dose, frequency, waste disposal - Laboratory experiments: **host range, infectivity and pathogenicity/virulence, potential for reproduction and gene transmission**, survival time outside the host - Animal studies: **tissue tropism**, **biodistribution**, latency/reactivation in the host, **shedding data** - Clinical trials: **shedding data** the potential to compete with existing species, ,

		ingestion, surface contact, burrowing, etc., 7. description of ecosystems to which the GMOs could be disseminated,	
2.	Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).	**THE GMO** A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s) B. Characteristics of the vector C. Characteristics of the modified organism 1. Information relating to the genetic modification: 2. Information on the final GMO (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed; (e) activity of the expressed protein(s); (h) history of previous releases or uses of the GMO; **CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT** A. Information on the release B. Information on the environment (both on the site and in the wider environment): **THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT** B. Interactions with the environment 4. likelihood of postrelease selection leading to the expression of unexpected and/or undesirable traits in the modified organism, 6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc., 7. description of ecosystems to which the GMOs could be disseminated, 8. potential for excessive population increase in the environment, 9. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s),	- Literature study: on donor, the recipient or parental organism. - Data on vector/GMO production, purification and verification (qPCR assay, bio-assay) - Molecular characterization: genetic stability, **characterization of the newly expressed protein** - Treatment data: dose, frequency, waste disposal - Laboratory experiments: **host range, infectivity and pathogenicity/virulence, potential for reproduction and gene transmission,** survival time outside the host - Animal studies: **tissue tropism**, biodistribution, **shedding data** - Clinical trials: **shedding data**
3.	Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage	**THE GMO** A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s) B. Characteristics of the vector	- Literature study: on donor, the recipient or parental organism: characteristics, **prevalence**. - Data on **vector/GMO production,**

	conferred to those species.	C. Characteristics of the modified organism 1. Information relating to the genetic modification: 2. Information on the final GMO (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed; (c) stability of the organism in terms of genetic traits; (h) history of previous releases or uses of the GMO; **CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT** A. Information on the release B. Information on the environment (both on the site and in the wider environment): **THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT** B. Interactions with the environment 3. genetic transfer capability	**purification and verification** (qPCR assay, bio-assay) - Molecular characterization: prokaryotic promoters, **homologous sequences**, mobile elements, the presence of antibiotic resistance genes, **genome integration sites** - Treatment data: **dose, frequency**, waste disposal - Literature study: **presence of recipient micro-organisms**, - Animal studies: **tissue tropism, biodistribution**, **shedding data** (qPCR assay, infectivity assays), **survival time outside the host** - Clinical trials: **shedding data** (qPCR assay, infectivity assays), **survival time outside the host**
4.	Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).	not applicable for human gene therapy applications	
5.	Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.	**THE GMO** A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s) B. Characteristics of the vector C. Characteristics of the modified organism 1. Information relating to the genetic modification: 2. Information on the final GMO (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed; (d) rate and level of expression of the new genetic material. Method and sensitivity of measurement; (e) activity of the expressed protein(s); (h) history of previous releases or uses of the GMO; (i) considerations for human health and animal health, as well as plant health:	- Literature study: on donor, the recipient or parental organism: **characteristics, prevalence** - Data on vector/GMO production, purification and verification (qPCR assay, bio-assay); characteristics of the insert - Molecular characterization: genetic map, sequence analysis, **replication competence**, **presence of genome integration sites**, genetic stability, potential for reversion to virulence - Treatment data: dose, frequency, waste disposal - Desk study; **toxigenicity and allergenicity**, - Laboratory studies: **toxigenicity and allergenicity**, apoptosis, extracellular and

		CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT A. Information on the release B. Information on the environment (both on the site and in the wider environment): **THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT** B. Interactions with the environment 7. description of ecosystems to which the GMOs could be disseminated, 8. potential for excessive population increase in the environment, 9. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s), 12. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction, 13. likelihood of postrelease shifts in biological interactions or in host range 14. known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens,	intracellular signalling, **pathogenicity/virulence**, expression levels, survival time outside the host - Animal studies: infectivity, pathogenicity, tissue tropism, **biodistribution**, expression levels, **shedding data** (qPCR assay, infectivity assays), **immunomodulation**, - Clinical trials: **shedding data** (qPCR assay, infectivity assays)
6.	Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).	**THE GMO** A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s) B. Characteristics of the vector C. Characteristics of the modified organism 1. Information relating to the genetic modification: 2. Information on the final GMO (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed; (d) rate and level of expression of the new genetic material. Method and sensitivity of measurement; (e) activity of the expressed protein(s); (h) history of previous releases or uses of the GMO; (i) considerations for human health and animal health, as well as plant health:	- Literature study: on donor, the recipient or parental organism: **characteristics, prevalence** - Data on vector/GMO production, purification and verification (qPCR assay, bio-assay); characteristics of the insert - Molecular characterization: genetic map, sequence analysis, **replication competence, presence of genome integration sites**, genetic stability, potential for reversion to virulence - Treatment data: dose, frequency, waste disposal - Desk study; **toxigenicity and allergenicity**, - Laboratory studies: **toxigenicity and allergenicity**, apoptosis, extracellular and

		CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT A. Information on the release B. Information on the environment (both on the site and in the wider environment): **THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT** A. Characteristics affecting survival, multiplication and dissemination 3. sensitivity to specific agents. B. Interactions with the environment 3. genetic transfer capability 6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc., 12. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction,	intracellular signalling, **pathogenicity/virulence**, expression levels, survival time outside the host - Animal studies: infectivity, pathogenicity, **tissue tropism**, **biodistribution** (qPCR assay), expression levels, **shedding data** (qPCR assay, infectivity assays), **immunomodulation**, - Clinical trials: **shedding data** (qPCR assay, infectivity assays)
7.	Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.	not applicable for human gene therapy applications	
8.	Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).	**THE GMO** A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s) B. Characteristics of the vector C. Characteristics of the modified organism 1. Information relating to the genetic modification: 2. Information on the final GMO (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed; (d) rate and level of expression of the new genetic material. Method and sensitivity of measurement; (e) activity of the expressed protein(s); (h) history of previous releases or uses of the GMO; **CONDITIONS OF RELEASE AND THE RECEIVING**	- Literature study: on donor, the recipient or parental organism. - Data on vector/GMO production, purification and verification (qPCR assay, bio-assay); characteristics of the insert - Molecular characterization: **replication competence, presence of genome integration sites** - Treatment data: waste disposal - Laboratory studies: **pathogenicity/virulence**, expression levels, survival time outside the host - Animal studies: expression levels, biodistribution, shedding data, - Clinical trials: shedding data

		ENVIRONMENT A. Information on the release B. Information on the environment (both on the site and in the wider environment): **THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT** B. Interactions with the environment 7. description of ecosystems to which the GMOs could be disseminated, 8. potential for excessive population increase in the environment 9. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s), 12. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction, 13. likelihood of postrelease shifts in biological interactions or in host range 14. known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens, 15. known or predicted involvement in biogeochemical processes,	
9.	Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.	**THE GMO** A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s) C. Characteristics of the modified organism 1. Information relating to the genetic modification: 2. Information on the final GMO (a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed; (e) activity of the expressed protein(s);	- Literature study: disease profile, prophylaxis and treatment of the parental organism - Data on the characteristics of the insert

Annex 5 Research related to application dossiers for market introduction of GM plants

Table 1 List of sources for ERA reports on commercialised GM plants

Country	Website
EU	http://www.gmo-compass.org/eng/gmo/db/ and http://ec.europa.eu/food/plant/gmo/new/eu_register/index_en.htm
the Netherlands	http://www.cogem.net/index.cfm/nl/publicaties/categorie/advies
Australia	http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/cr-1
Canada	http://www.inspection.gc.ca/plants/plants-with-novel-traits/approved-under-review/decision-documents/eng/1303704378026/1303704484236
China	Only a list of commercialised products is published http://english.biosafety.gov.cn/html/gene_sql.htm
USA	http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml

Table 2 GM plant events and stacks approved for commercialisation

Source: International Service for the Acquisition of Agri-biotech Applications (ISAAA) GM approval database[97]

Abbreviations: AS: Abiotic stress, PB: Plant biology, HT: Herbicide tolerance, IR: Insect resistance, VR: Virus resistance, PQ: Product quality, PS: Product systems, MG: Marker genes
*: only authorised for food and/or feed
**: environmental certificate issued

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Alfalfa	*Medicago sativa*	**J101**	MON-ØØ1Ø1-8	Monsanto and Forage Genetics	2004 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Alfalfa	*Medicago sativa*	**J101 x J163**	MON-ØØ1Ø1-8 x MON-ØØ163-7	Monsanto and Forage Genetics	2005 (Japan)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Alfalfa	*Medicago sativa*	**J163**	MON-ØØ163-7	Monsanto and Forage Genetics	2004 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Alfalfa	*Medicago sativa*	**KK179**	MON-ØØ179-5	Monsanto and Forage Genetics	2013 (USA)	0	0	0	0	0	X	0	X	Altered lignin production
Alfalfa	*Medicago sativa*	**KK179 x J101**	MON-ØØ179-5 x MON-ØØ1Ø1-8	Monsanto	2015 (Mexico)	0	0	X	0	0	X	0	X	Glyphosate tolerance, Altered lignin production

[97] http://www.isaaa.org/gmapprovaldatabase/default.asp

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Apple	*Malus domestica*	**GD743**	OKA-NBØØ1-8	Okanagan Specialty Fruits	2015 (Canada, USA)	0	0	0	0	0	X	0	X	Non-browning
Apple	*Malus domestica*	**GD784**	OKA-NBØØ2-9	Okanagan Specialty Fruits	2015 (Canada, USA)	0	0	0	0	0	X	0	X	Non-browning
Bean	*Phaseolus vulgaris*	**EMBRAPA 5.1**	EMB-PVØ51-1	EMBRAPA (Brazil)	2011 (Brazil)	0	0	0	0	X	0	0	0	Bean Golden Mosaic Virus resistance
Carnation	*Dianthus caryophillus*	**4**	FLO-ØØØØ4-9	Florigene	1995 (Australia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**15**	FLO-ØØØ15-2	Florigene	1995 (Australia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**16**	FLO-ØØØ16-3	Florigene	1995 (Australia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**66**	FLO-ØØØ66-8	Florigene	1995 (Australia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Increased shelf-life
Carnation	*Dianthus caryophillus*	**25947**	IFD-25947-1	Suntory Limited (Japan)	2008 (Colombia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**25958**	IFD-25958-3	Suntory Limited (Japan)	2008 (Colombia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**11 (7442)**	FLO-Ø7442-4	Florigene	1995 (Australia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**11363 (1363A)**	FLO-11363-1	Florigene	1998 (EU, Norway)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**1226A (11226)**	FLO-11226-8	Florigene	1998 (EU, Norway)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**123.2.2 (40619)**	FLO-4Ø619-7	Florigene	2004 (Japan)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**123.2.38 (40644)**	FLO-4Ø644-4	Florigene	2004 (Japan)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**123.8.12**	FLO-4Ø689-6	Florigene	2009 (Japan)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**123.8.8 (40685)**	FLO-4Ø685-1	Florigene	2004 (Japan)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**1351A (11351)**	FLO-11351-7	Florigene	1998 (Norway)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**1400A (11400)**	FLO-114ØØ-2	Florigene	1998 (Norway)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus*	**199Ø7**	IFD-199Ø7-9	Suntory Limited	2008 (Colombia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance ,

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
	caryophillus			(Japan)										Modified flower color
Carnation	*Dianthus caryophillus*	**264Ø7**	IFD-264Ø7-2	Suntory Limited (Japan)	2008 (Colombia)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**959A (11959)**	FLO-11959-3	Florigene	1998 (EU, Norway)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Carnation	*Dianthus caryophillus*	**988A (11988)**	FLO-11988-7	Florigene	1998 (EU, Norway)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance , Modified flower color
Chicory	*Cichorium intybus*	**RM3-3**	not available	Bejo Zaden	1997 (USA)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Male sterility
Chicory	*Cichorium intybus*	**RM3-4**	not available	Bejo Zaden	1997 (USA)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Male sterility
Chicory	*Cichorium intybus*	**RM3-6**	not available	Bejo Zaden	1997 (USA)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Male sterility
Cotton	*Gossypium hirsutum*	**31707**	not available	Monsanto	1998 (USA)*	0	0	X	X	0	0	0	X	Oxynil tolerance , Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**31803**	not available	Monsanto	1998 (USA)*	0	0	X	X	0	0	0	X	Oxynil tolerance , Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**31808**	not available	Monsanto	1997 (USA)	0	0	X	X	0	0	0	X	Oxynil tolerance , Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**42317**	not available	Monsanto	1998 (USA)*	0	0	X	X	0	0	0	X	Oxynil tolerance , Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**81910**	DAS-81910-7	Dow AgroSciences	2014 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance , 2,4-D tolerance
Cotton	*Gossypium hirsutum*	**19-51a**	DD-Ø1951A-7	DuPont (Pioneer Hi-Bred)	1996 (USA)	0	0	X	0	0	0	0	0	Sulfonylurea tolerance
Cotton	*Gossypium hirsutum*	**281-24-236**	DAS-24236-5	Dow AgroSciences	2004 (Mexico, USA)	0	0	X	X	0	0	0	0	Lepidopteran resistance, Glufosinate ammonium tolerance
Cotton	*Gossypium hirsutum*	**281-24-236 x 3006-210-23**	DAS-24236-5 x DAS-21Ø23-5	Dow AgroSciences	2004 (Mexico)	0	0	X	X	0	0	0	0	Lepidopteran resistance, Glufosinate ammonium tolerance
Cotton	*Gossypium hirsutum*	**3006-210-23**	DAS-21Ø23-5	Dow AgroSciences	2004 (Mexico, USA)	0	0	X	X	0	0	0	0	Lepidopteran resistance, Glufosinate ammonium tolerance
Cotton	*Gossypium hirsutum*	**3006-210-23 x 281-24-236 x MON1445**	DAS-21Ø23-5 x DAS-24236-5 x MON-Ø1445-2	Monsanto and Dow AgroSciences	2005 (Mexico)	0	0	X	X	0	0	0	X	Lepidopteran resistance, Glufosinate ammonium tolerance, Glyphosate

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
														tolerance
Cotton	*Gossypium hirsutum*	**3006-210-23 x 281-24-236 x MON88913**	DAS-21Ø23-5 x DAS-24236-5 x MON-88913-8	Monsanto and Dow AgroSciences	2006 (Japan, Mexico, South Korea)	0	0	X	X	0	0	0	0	Lepidopteran resistance, Glufosinate ammonium tolerance, Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**3006-210-23 x 281-24-236 x MON88913 x COT102**	DAS-21Ø23-5 x DAS-24236-5 x MON-88913-8 x SYN-IR1Ø2-7	Dow AgroSciences	2014 (Mexico, South Korea)	0	0	X	X	0	0	0	X	Lepidopteran resistance, Glufosinate ammonium tolerance, Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**31807 x 31808**	not available	Monsanto	1998 (Canada)	0	0	X	X	0	0	0	X	Oxynil tolerance , Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**31807**	not available	Monsanto	1997 (USA)	0	0	X	X	0	0	0	0	Oxynil tolerance , Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**BNLA-601**	not available	Central Institute for Cotton Research and University of Agricultural Sciences Dharwad (India)	2008 (India)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**BXN10211 (10211)**	BXN-1Ø211-9	Monsanto	1994 (USA)	0	0	X	0	0	0	0	X	Oxynil tolerance
Cotton	*Gossypium hirsutum*	**BXN10215 (10215)**	BXN-1Ø215-4	Monsanto	1994 (USA)	0	0	X	0	0	0	0	X	Oxynil tolerance
Cotton	*Gossypium hirsutum*	**BXN10222 (10222)**	BXN-1Ø222-2	Monsanto	1994 (USA)	0	0	X	0	0	0	0	X	Oxynil tolerance
Cotton	*Gossypium hirsutum*	**BXN10224 (10224)**	BXN-1Ø224-4	Monsanto	1994 (USA)	0	0	X	0	0	0	0	X	Oxynil tolerance
Cotton	*Gossypium hirsutum*	**COT102 (IR102)**	SYN-IR1Ø2-7	Syngenta	2005 (Australia, New Zealand, USA)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**COT102 x COT67B**	SYN-IR1Ø2-7 x SYN-IR67B-1	Syngenta	2009 (Costa Rica)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**COT102 x COT67B x MON88913**	SYN-IR1Ø2-7 x SYN-IR67B-1 x MON-88913-8	Syngenta and Monsanto	2009 (Costa Rica)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**COT102 x MON15985**	SYN-IR1Ø2-7 x MON-15985-7	Monsanto	2014 (Australia, Japan, Mexico)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**COT102 x MON15985 x MON88913**	SYN-IR1Ø2-7 x MON-15985-7 x MON-88913-8	Syngenta and Monsanto	2014 (Australia, Japan)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Cotton	*Gossypium hirsutum*	**COT67B (IR67B)**	SYN-IR67B-1	Syngenta	2009 (Australia, New Zealand, USA)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**Event1**	not available	JK Agri Genetics Ltd (India)	2006 (India)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**GFM Cry1A**	GTL-GFM311-7	Nath Seeds/Global Transgenes Ltd (India)	2006 (India)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**GHB119**	BCS-GHØØ5-8	Bayer CropScience	2011 (Australia, Canada, New Zealand, USA)	0	0	X	X	0	0	0	0	Lepidopteran resistance, Glufosinate ammonium tolerance
Cotton	*Gossypium hirsutum*	**GHB614**	BCS-GHØØ2-5	Bayer CropScience	2008 (Canada, Mexico)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**GHB614 x LLCotton25**	BCS-GHØØ2-5 x ACS-GHØØ1-3	Bayer CropScience	2010 (Japan, Mexico)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**GHB614 x LLCotton25 x MON15985**	BCS-GHØØ2-5 x ACS-GHØØ1-3 x MON-15985-7	Bayer CropScience	2010 (Japan, Mexico)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**GHB614 x MON15985**	BCS-GHØØ2-5 × MON-15985-7	Bayer CropScience	2010 (Japan)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**GHB614 x T304-40 x GHB119**	BCS-GHØØ2-5 x BCS-GHØØ4-7 x BCS-GHØØ5-8	Bayer CropScience	2012 (Brazil, Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**GHB614 x T304-40 x GHB119 x COT102**	BCS-GHØØ2-5 x BCS-GHØØ4-7 x BCS-GHØØ5-8 x SYN-IR1Ø2-7	Bayer CropScience	2015 (South Korea)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**GK12**	not available	Chinese Academy of Agricultural Sciences	1997 (China)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**LLCotton 25**	ACS-GHØØ1-3	Bayer CropScience	2003 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Cotton	*Gossypium hirsutum*	**LLCotton25 x MON15985**	ACS-GHØØ1-3 x MON-15985-7	Bayer CropScience	2006 (Australia, Japan, New Zealand, South Korea)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**MLS 9124**	not available	Metahelix Life Sciences Pvt. Ltd	2009 (India)	0	0	0	X	0	0	0	X	Lepidopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
				(India)										
Cotton	*Gossypium hirsutum*	**MON1076**	MON-89924-2	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**MON1445**	MON-Ø1445-2	Monsanto	1995 (USA)	0	0	X	0	0	0	0	X	Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**MON15985**	MON-15985-7	Monsanto	2002 (Australia, EU, Japan, New Zealand, USA)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**MON15985 x MON1445**	MON-15985-7 x MON-Ø1445-2	Monsanto	2002 (Australia, EU, New Zealand)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**MON1698**	MON-89383-1	Monsanto	1995 (USA)	0	0	X	0	0	0	0	X	Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**MON531**	MON-ØØ531-6	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**MON531 x MON1445**	MON-Ø531-6 x MON-Ø1445-2	Monsanto	2000 (New Zealand)	0	0	X	X	0	0	0	X	Lepidopteran resistance, Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**MON757**	MON-ØØ757-7	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**MON88701**	MON 887Ø1-3	Monsanto	2013 (Japan, USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance, Dicamba tolerance
Cotton	*Gossypium hirsutum*	**MON88701 x MON88913**	MON 887Ø1-3 x MON-88913-8	Monsanto	2015 (Japan, Mexico)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Dicamba tolerance
Cotton	*Gossypium hirsutum*	**MON88701 x MON88913 x MON15985**	MON 887Ø1-3 x MON-88913-8 x MON-15985-7	Monsanto	2014 (Mexico)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Dicamba tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**MON88913**	MON-88913-8	Monsanto	2004 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**MON88913 x MON15985**	MON-88913-8 x MON-15985-7	Monsanto	2005 (Japan)	0	0	X	X	0	0	0	X	Lepidopteran resistance, Glyphosate tolerance
Cotton	*Gossypium hirsutum*	**Ngwe Chi 6 Bt**	not available	Cotton and Sericulture Department (Myanmar)	2006 (Myanmar)	0	0	0	X	0	0	0	0	Lepidopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Cotton	*Gossypium hirsutum*	**SGK321**	not available	Chinese Academy of Agricultural Sciences	1999 (China)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**T303-3**	BCS-GHØØ3-6	Bayer CropScience	2012 (USA)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**T304-40**	BCS-GHØØ4-7	Bayer CropScience	2010 (Australia, New Zealand)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Cotton	*Gossypium hirsutum*	**T304-40 x GHB119**	BCS-GHØØ4-7 x BCS-GHØØ5-8	Bayer CropScience	2011 (Brazil, Canada))	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Creeping Bentgrass	*Agrostis stolonifera*	**ASR368**	SMG-368ØØ-2	Monsanto	2003 (USA)*	0	0	X	0	0	0	0	0	Glyphosate tolerance
Eggplant	*Solanum melongena*	**Bt Brinjal Event EE1**	not available	Maharashtra Hybrid Seed Company (MAHYCO)	2013 (Bangladesh)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Eucalyptus	*Eucalyptus sp.*	**H421**	not available	FuturaGene Group	2015 (Brazil)	0	X	0	0	0	0	0	X	Increased growth
Flax	*Linum usitatissumum*	**FP967 (CDC Triffid)**	CDC-FLØØ1-2	University of Saskatchewan	1996 (Canada)	0	0	X	0	0	0	0	X	Sulfonylurea tolerance
Maize	*Zea mays*	**676**	PH-ØØØ676-7	DuPont (Pioneer Hi-Bred)	1998 (USA)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility
Maize	*Zea mays*	**678**	PH-ØØØ678-9	DuPont (Pioneer Hi-Bred)	1998 (USA)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility
Maize	*Zea mays*	**680**	PH-ØØØ68Ø-2	DuPont (Pioneer Hi-Bred)	1998 (USA)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility
Maize	*Zea mays*	**3272**	SYN-E3272-5	Syngenta	2007 (USA)	0	0	0	0	0	X	0	X	Modified alpha amylase
Maize	*Zea mays*	**4114**	DP-ØØ4114-3	DuPont (Pioneer Hi-Bred)	2013 (Canada, USA)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**5307**	SYN-Ø53Ø7-1	Syngenta	2012 (Australia, Japan, New Zealand, Taiwan, USA)	0	0	0	X	0	0	0	X	Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**32138**	DP-32138-1	DuPont (Pioneer Hi-Bred)	2011 (USA)	0	X	0	0	0	0	0	X	Fertility restoration, Male sterility
Maize	*Zea mays*	**33121**	DP-Ø33121-3	DuPont (Pioneer Hi-Bred)	2014 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
														resistance
Maize	*Zea mays*	**59122**	DAS-59122-7	Dow AgroSciences and DuPont (Pioneer Hi-Bred)	2004 (Mexico, USA)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance
Maize	*Zea mays*	**98140**	DP-Ø9814Ø-6	DuPont (Pioneer Hi-Bred)	2008 (Mexico, USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance, Sulfonylurea tolerance
Maize	*Zea mays*	**3272 x Bt11**	SYN-E3272-5 x SYN-BTØ11-1	Syngenta	2010 (Japan)	0	0	X	X	0	X	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance, Modified alpha amylase
Maize	*Zea mays*	**3272 x Bt11 x GA21**	SYN-E3272-5 x SYN-BTØ11-1 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	X	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance, Modified alpha amylase
Maize	*Zea mays*	**3272 x Bt11 x MIR604**	SYN-E3272-5 x SYN-BTØ11-1 x SYN-IR6Ø4-5	Syngenta	2010 (Japan)	0	0	X	X	0	X	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance, Modified alpha amylase
Maize	*Zea mays*	**3272 x BT11 x MIR604 x GA21**	SYN-E3272-5 x SYN-BTØ11-1 x SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2010 (Japan, Mexico, Philippines)	0	0	X	X	0	X	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance, Modified alpha amylase
Maize	*Zea mays*	**3272 x Bt11 x MIR604 x TC1507 x 5307 x GA21**	SYN-E3272-5 x SYN-BTØ11-1 x SYN-IR6Ø4-5 x DAS-Ø15Ø7-1 x SYN-Ø53Ø7-1 x MON-ØØØ21-9	Syngenta	2014 (South Korea)	0	0	X	X	0	X	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance, Modified alpha amylase
Maize	*Zea mays*	**3272 x GA21**	SYN-E3272-5 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	0	0	X	0	X	Glyphosate tolerance, Modified alpha amylase
Maize	*Zea mays*	**3272 x MIR604**	SYN-E3272-5 x SYN-IR6Ø4-5	Syngenta	2010 (Japan)	0	0	0	X	0	X	0	X	Coleopteran resistance, Modified alpha amylase
Maize	*Zea mays*	**3272 x MIR604 x GA21**	SYN-E3272-5 x SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	X	0	X	Glyphosate tolerance, Coleopteran resistance, Modified alpha amylase

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Maize	*Zea mays*	**5307 x MIR604 x Bt11 x TC1507 x GA21**	SYN-Ø53Ø7-1 x SYN-IR6Ø4-5 x SYN-BTØ11-1 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2013 (Canada, Japan, Mexico, Taiwan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**5307 x MIR604 x Bt11 x TC1507 x GA21 x MIR162**	SYN-Ø53Ø7-1 x SYN-IR6Ø4-5 x SYN-BTØ11-1 x DAS-Ø15Ø7-1 x MON-ØØØ21-9 x SYN-IR162-4	Syngenta	2013 (Canada, Japan, Mexico, Taiwan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**59122 x GA21**	DAS-59122-7 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance
Maize	*Zea mays*	**59122 x MIR604**	DAS-59122-7 x SYN-IR6Ø4-5	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance
Maize	*Zea mays*	**59122 x MIR604 x GA21**	DAS-59122-7 x SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance
Maize	*Zea mays*	**59122 x MIR604 x TC1507**	DAS-59122-7 x SYN-IR6Ø4-5 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, lepidopteran resistance
Maize	*Zea mays*	**59122 x MIR604 x TC1507 x GA21**	DAS-59122-7 x SYN-IR6Ø4-5 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, lepidopteran resistance
Maize	*Zea mays*	**59122 x MON810**	DAS-59122-7 x MON-ØØ81Ø-6	DuPont (Pioneer Hi-Bred)	2009 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, lepidopteran resistance
Maize	*Zea mays*	**59122 x MON810 x NK603**	DAS-59122-7 x MON-ØØ81Ø-6 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2009 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Maize	*Zea mays*	**59122 x MON88017**	DAS-59122-7 x MON-88Ø17-3	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance
Maize	*Zea mays*	**59122 x NK603**	DAS-59122-7 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2005 (Canada, Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance
Maize	*Zea mays*	**59122 x TC1507 x GA21**	DAS-59122-7 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**98140 x 59122**	DP-Ø9814Ø-6 x DAS-59122-7	Dow AgroSciences and DuPont (Pioneer Hi-Bred)	2010 (Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Sulfonylurea tolerance, Coleopteran resistance
Maize	*Zea mays*	**98140 x TC1507**	DP-Ø9814Ø-6 x DAS-Ø15Ø7-1	Dow AgroSciences and DuPont (Pioneer Hi-Bred)	2010 (Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Sulfonylurea tolerance, Lepidopteran resistance
Maize	*Zea mays*	**98140 x TC1507 x 59122**	DP-Ø9814Ø-6 x DAS-Ø15Ø7-1 x DAS-59122-7	Dow AgroSciences and DuPont (Pioneer Hi-Bred)	2010 (Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Sulfonylurea tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt10**	not available	Syngenta	2007 (South Korea)*	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 (X4334CBR, X4734CBR)**	SYN-BTØ11-1	Syngenta	2001 (Argentina, Australia, Japan, New Zealand)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x 59122**	SYN-BTØ11-1 x DAS-59122-7	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x 59122 x**	SYN-BTØ11-1 x	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
		GA21	DAS-59122-7 x MON-ØØØ21-9											tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x 59122 x MIR604**	SYN-BTØ11-1 x DAS-59122-7 x SYN-IR6Ø4-5	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x 59122 x MIR604 x GA21**	SYN-BTØ11-1 x DAS-59122-7 x SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x 59122 x MIR604 x TC1507**	SYN-BTØ11-1 x DAS-59122-7 x SYN-IR6Ø4-5 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**BT11 x 59122 x MIR604 x TC1507 x GA21**	SYN-BTØ11-1 x DAS-59122-7 x SYN-IR6Ø4-5 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2010 (Canada, Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x 59122 x TC1507**	SYN-BTØ11-1 x DAS-59122-7 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x 59122 x TC1507 x GA21**	SYN-BTØ11-1 x DAS-59122-7 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x GA21**	SYN-BTØ11-1 x MON-ØØØ21-9	Syngenta	2005 (Canada)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x MIR162**	SYN-BTØ11-1 x SYN-IR162-4	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x MIR162 x GA21**	SYN-BTØ11-1 x SYN-IR162-4 x	Syngenta	2010 (Brazil, Colombia, Japan,	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
			MON-ØØØ21-9		Mexico, Philippines)									tolerance, Lepidopteran resistance
Maize	*Zea mays*	**BT11 x MIR162 x MIR604**	SYN-BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x MIR162 x MIR604 x GA21**	SYN-BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2010 (Canada, Japan, Mexico, Philippines, South Korea)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x MIR162 x TC1507**	SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x MIR162 x TC1507 x GA21**	SYN-BTØ11-1 x SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2010 (Canada, Japan,Philippines)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x MIR604**	SYN-BTØ11-1 x SYN-IR6Ø4-5	Syngenta	2007 (Canada, Japan, Mexico, Philippines, South Korea)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**BT11 x MIR604 x GA21**	SYN-BTØ11-1 x SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2007 (Canada, Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x MIR604 x TC1507**	SYN-BTØ11-1 x SYN-IR6Ø4-5 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x TC1507**	SYN-BTØ11-1 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt11 x TC1507 x GA21**	SYN-BTØ11-1 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**Bt176 (176)**	SYN-EV176-9	Syngenta	1995 (USA)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
														resistance
Maize	*Zea mays*	**BVLA430101**	not available	Origin Agritech (China)	2009 (China)	0	0	0	0	0	X	0	0	Phytase production
Maize	*Zea mays*	**CBH-351**	ACS-ZMØØ4-3	Bayer CropScience	1998 (USA)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**DAS40278**	DAS-4Ø278-9	Dow AgroSciences	2011 (Australia, Mexico, New Zealand, Taiwan, USA)	0	0	X	0	0	0	0	0	2,4-D herbicide tolerance
Maize	*Zea mays*	**DAS40278 x NK603**	DAS-4Ø278-9 x MON-ØØ6Ø3-6	Dow AgroSciences	2013 (Canada, Japan, Mexico, Taiwan)	0	0	X	0	0	0	0	0	Glyphosate tolerance, 2,4-D herbicide tolerance
Maize	*Zea mays*	**DBT418**	DKB-89614-9	Monsanto	1997 (Canada, USA)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**DLL25 (B16)**	DKB-8979Ø-5	Monsanto	1995 (USA)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance
Maize	*Zea mays*	**GA21**	MON-ØØØ21-9	Monsanto	1997 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Maize	*Zea mays*	**GA21 x MON810**	MON-ØØØ21-9 x MON-ØØ81Ø-6	Monsanto	EU (1998)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**GA21 x T25**	MON-ØØØ21-9 x ACS-ZMØØ3-2	Syngenta	2014 (Philippines, South Korea, Taiwan)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance
Maize	*Zea mays*	**HCEM485**	not available	Stine Seed Farm, Inc (USA)	2012 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Maize	*Zea mays*	**LY038**	REN-ØØØ38-3	Renessen LLC (Netherlands)	2003 (Taiwan)	0	0	0	0	0	X	0	0	Modified amino acid
Maize	*Zea mays*	**LY038 x MON810**	REN-ØØØ38-3 x MON-ØØ81Ø-6	Renessen LLC (Netherlands)	2007 (Japan)	0	0	0	X	0	X	0	0	Modified amino acid, Lepidopteran resistance
Maize	*Zea mays*	**MIR162**	SYN-IR162-4	Syngenta	2008 (USA)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Maize	*Zea mays*	**MIR162 x GA21**	SYN-IR162-4 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MIR162 x MIR604**	SYN-IR162-4 x SYN-IR6Ø4-5	Syngenta	2010 (Japan)	0	0	0	X	0	0	0	X	Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MIR162 x MIR604 x GA21**	SYN-IR162-4 x SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Maize	*Zea mays*	**MIR162 x NK603**	SYN-IR162-4 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2015 (Brazil)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MIR162 x TC1507**	SYN-IR162-4 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MIR162 x TC1507 x GA21**	SYN-IR162-4 x DAS-Ø15Ø7-1 x MON-ØØØ21-9	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MIR604**	SYN-IR6Ø4-5	Syngenta	2006 (Australia, New Zealand)	0	0	0	X	0	0	0	X	Coleopteran resistance
Maize	*Zea mays*	**MIR604 x GA21**	SYN-IR6Ø4-5 x MON-ØØØ21-9	Syngenta	2007 (Japan, Mexico, Philippines)	0	0	X	X	0	0	0	X	Glyphosate toleranceColeopteran resistance
Maize	*Zea mays*	**MIR604 x NK603**	SYN-IR6Ø4-5 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2011 (Japan)	0	0	X	X	0	0	0	X	Glyphosate toleranceColeopteran resistance
Maize	*Zea mays*	**MIR604 x TC1507**	SYN-IR6Ø4-5 x DAS-Ø15Ø7-1	Syngenta	2010 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON801 (MON80100)**	not available	Monsanto	1995 (USA)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MON802**	MON-8Ø2ØØ-7	Monsanto	1996 (USA)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MON809**	PH-MON-8Ø9-2	Monsanto and Dupont (Pioneer Hi-bred	1996 (USA)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MON810**	MON-ØØ81Ø-6	Monsanto	1995 (USA)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Maize	*Zea mays*	**MON810 x MIR162**	MON-ØØ81Ø-6 x SYN-IR162-4	DuPont (Pioneer Hi-Bred)	2015 (Brazil)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Maize	*Zea mays*	**MON810 x MON88017**	MON-ØØ81Ø-6 x MON-88Ø17-3	Monsanto	2005 (Japan)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON832**	not available	Monsanto	1996 (USA)*	0	0	X	0	0	0	0	X	Glyphosate tolerance
Maize	*Zea mays*	**MON863**	MON-ØØ863-5	Monsanto	2001 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Maize	*Zea mays*	**MON863 x MON810**	MON-ØØ863-5 x MON-ØØ81Ø-6	Monsanto	2004 (Japan, Philippines, South Korea)	0	0	0	X	0	0	0	X	Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON863 x MON810 x NK603**	MON-ØØ6Ø3-6 x MON-ØØ81Ø-6 x MON-ØØ863-5	Monsanto	2004 (Canada, Japan, South Korea)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON863 x NK603**	MON-ØØ863-5 x MON-ØØ6Ø3-6	Monsanto	2003 (Japan)	0	0	X	X	0	0	0	X	Glyphosate tolerance, Coleopteran resistance
Maize	*Zea mays*	**MON87411**	MON-87411-9	Monsanto	2014 (Japan, USA)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Coleopteran resistance
Maize	*Zea mays*	**MON87427**	MON-87427-7	Monsanto	2012 (Australia, Canada, Mexico, New Zealand, Taiwan, USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Maize	*Zea mays*	**MON87427 x MON89034 x MON88017**	MON-87427-7 x MON-89Ø34-3 x MON-88Ø17-3	Monsanto	2013 (Japan, Mexico)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON87427 x MON89034 x NK603**	MON-87427-7 x MON-89Ø34-3 x MON-ØØ6Ø3-6	Monsanto	2013 (Japan, Mexico)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MON87427 x MON89Ø34 x TC15Ø7 x MON88Ø17 x 59122**	MON-87427-7 x MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7	Monsanto	2013 (Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON87460**	MON-8746Ø-4	Monsanto	2010 (Australia, Canada, New Zealand, USA)	X	0	0	0	0	0	0	X	Drought tolerance
Maize	*Zea mays*	**MON87460 x MON89034 x MON88017**	MON-8746Ø-4 x MON-89Ø34-3 x MON-88Ø17-3	Monsanto	2012 (Japan, Mexico, Taiwan)	X	0	X	X	0	0	0	X	Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance, Drought tolerance
Maize	*Zea mays*	**MON87460 x MON89034 x NK603**	MON-8746Ø-4 x MON-89Ø34-3 x MON-ØØ6Ø3-6	Monsanto	2012 (Japan, Mexico, South Korea, Taiwan)	X	0	X	X	0	0	0	X	Glyphosate tolerance, Lepidopteran resistance, Drought tolerance
Maize	*Zea mays*	**MON87460 x NK603**	MON-8746Ø-4 x MON-ØØ6Ø3-6	Monsanto	2012 (Japan, Mexico, South Korea, Taiwan)	X	0	X	0	0	0	0	X	Glyphosate tolerance, Drought tolerance
Maize	*Zea mays*	**MON88017**	MON-88Ø17-3	Monsanto	1995 (USA)	0	0	X	X	0	0	0	0	Glyphosate tolerance,

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
														Coleopteran resistance
Maize	*Zea mays*	**MON89034**	MON-89Ø34-3	Monsanto	2007 (Colombia, Japan, USA)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x 59122**	MON-89Ø34-3 x DAS-59122-7	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x 59122 x MON88017**	MON-89Ø34-3 x DAS-59122-7 x MON-88Ø17-3	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x MON88017**	MON-89Ø34-3 x MON-88Ø17-3	Monsanto	2007 (Japan)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x NK603**	MON-89Ø34-3 x MON-ØØ6Ø3-6	Monsanto	2007 (Japan)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x TC1507**	MON-89Ø34-3 x DAS-Ø15Ø7-1	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x TC1507 x 59122**	MON-89Ø34-3 x DAS- Ø15Ø7-1 x DAS-59122-7	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x TC1507 x MON88017**	MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x TC1507 x MON88017 x 59122**	MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x TC1507 x MON88017 x 59122 x DAS40278**	MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7 x DAS-4Ø278-9	Dow AgroSciences	2013 (Canada, Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance, 2,4-D tolernace

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Maize	*Zea mays*	**MON89034 x TC1507 x MON88017 x DAS40278**	MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7 x DAS-4Ø278-9	Dow AgroSciences	2013 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance, 2,4-D tolernace
Maize	*Zea mays*	**MON89034 x TC1507 x NK603**	MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-ØØ6Ø3-6	Monsanto	2010 (Brazil, Japan, Philippines, South Korea)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**MON89034 x TC1507 x NK603 x DAS40278**	MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-ØØ6Ø3-6 x DAS-4Ø278-9	Dow AgroSciences	2013 (Japan, Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance, 2,4-D tolernace
Maize	*Zea mays*	**MS3**	ACS-ZMØØ1-9	Bayer CropScience	1996 (Canada, USA)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance , Male sterility
Maize	*Zea mays*	**MS6**	ACS-ZMØØ5-4	Bayer CropScience	1996 (USA)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance , Male sterility
Maize	*Zea mays*	**NK603**	MON-ØØ6Ø3-6	Monsanto	2000 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Maize	*Zea mays*	**NK603 x MON810**	MON-ØØ6Ø3-6 x MON-ØØ81Ø-6	Monsanto	2002 (Japan)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**NK603 x MON810 x 4114 x MIR 604**	MON-00603-6 x MON-00810-6 x DP004114-3 x SYN-IR604-4	Syngenta	2013 (Canada)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**NK603 x T25**	MON-ØØ6Ø3-6 x ACS-ZMØØ3-2	Monsanto	2009 (Japan)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance
Maize	*Zea mays*	**T14**	ACS-ZMØØ2-1	Bayer CropScience	1995 (USA)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance
Maize	*Zea mays*	**T25**	ACS-ZMØØ3-2	Bayer CropScience	1995 (USA)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance
Maize	*Zea mays*	**T25 x MON810**	ACS-ZMØØ3-2 x MON-ØØ81Ø-6	Monsanto	2001 (Japan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507**	DAS-Ø15Ø7-1	Dow AgroSciences	2001 (USA)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
														resistance
Maize	*Zea mays*	**TC1507 × 59122 × MON810 × MIR604 x NK603**	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ81Ø-6 × SYN-IR6Ø4-5 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2011 (Canada, Mexico)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 × MON810 × MIR604 × NK603**	DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR6Ø4-5 × MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2013 (Mexico)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x 59122**	DAS-Ø15Ø7-1 x DAS-59122-7	Dow AgroSciences	2005 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x 59122 x MON810**	DAS-Ø15Ø7-1 x DAS-59122-7 x MON-ØØ81Ø-6	DuPont (Pioneer Hi-Bred)	2009 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x 59122 x MON810 x NK603**	DAS-Ø15Ø7-1 x DAS-59122-7 x MON-ØØ81Ø-6 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2009 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x 59122 x MON88017**	DAS-Ø15Ø7-1 x DAS-59122-7 x MON-88Ø17-3	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x 59122 x NK603**	DAS-Ø15Ø7-1 x DAS-59122-7 x MON-ØØ6Ø3-6	Dow AgroSciences	2005 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x GA21**	DAS-Ø15Ø7-1 x MON-ØØØ21-9	DuPont (Pioneer Hi-Bred)	2010 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x MIR162 x NK603**	DAS-Ø15Ø7-1 x SYN-IR162-4 x	DuPont (Pioneer Hi-Bred)	2015 (Brazil, Mexico)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
			MON-ØØ6Ø3-6											tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x MIR604 x NK603**	DAS-Ø15Ø7-1 x SYN-IR6Ø4-5 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2011 (Canada, Japan, Mexico, Taiwan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x MON810**	DAS-Ø15Ø7-1 x MON-ØØ81Ø-6	Dow AgroSciences	2010 (Mexico)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x MON810 x MIR162**	DAS-Ø15Ø7-1 x MON-ØØ81Ø-6 x SYN-IR162-4	DuPont (Pioneer Hi-Bred)	2015 (Brazil, Mexico, South Korea, Taiwan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x MON810 x MIR162 x NK603**	DAS-Ø15Ø7-1 x MON-ØØ81Ø-6 x SYN-IR162-4 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2013 (Japan, Mexico, South Korea, Taiwan)	0	0	X	X	0	0	0	X	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x MON810 x NK603**	DAS-Ø15Ø7-1 x MON-ØØ81Ø-6 x MON-ØØ6Ø3-6	DuPont (Pioneer Hi-Bred)	2009 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x MON88017**	DAS-Ø15Ø7-1 x MON-88Ø17-3	Monsanto	2008 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Coleopteran resistance, Lepidopteran resistance
Maize	*Zea mays*	**TC1507 x NK603**	DAS-Ø15Ø7-1 x MON-ØØ6Ø3-6	Dow AgroSciences		0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Lepidopteran resistance
Maize	*Zea mays*	**TC6275**	DAS-Ø6275-8	Dow AgroSciences	2003 (USA)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Maize	*Zea mays*	**VCO-Ø1981-5**	VCO-Ø1981-5	Genective S.A.	2013 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Melon	*Cucumis melo*	**Melon A**	not available	Agritope Inc. (USA)	1999 (USA)*	0	0	0	0	0	X	0	X	Delayed ripening/senescence
Melon	*Cucumis melo*	**Melon B**	not available	Agritope Inc. (USA)	1999 (USA)*	0	0	0	0	0	X	0	X	Delayed ripening/senescence
Oilseed rape	*Brassica napus*	**61061**	DP-Ø61Ø61-7	DuPont (Pioneer Hi-	2012 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
				Bred)										
Oilseed rape	*Brassica napus*	**73496**	DP-Ø73496-4	DuPont (Pioneer Hi-Bred)	2012 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Oilseed rape	*Brassica napus*	**23-18-17 (Event 18)**	CGN-89111-8	Monsanto	1994 (USA)	0	0	0	0	0	X	0	X	Modified oil/fatty acid
Oilseed rape	*Brassica napus*	**23-198 (Event 23)**	CGN-89465-2	Monsanto	1994 (USA)	0	0	0	0	0	X	0	X	Modified oil/fatty acid
Oilseed rape	*Brassica napus*	**GT200 (RT200)**	MON-89249-2	Monsanto	1997 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Oilseed rape	*Brassica napus*	**GT73 (RT73)**	MON-ØØØ73-7	Monsanto	1994 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Oilseed rape	*Brassica napus*	**HCN10 (Topas 19/2)**	not available	Bayer CropScience	1995 (USA)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance
Oilseed rape	*Brassica napus*	**HCN28 (T45)**	ACS-BNØØ8-2	Bayer CropScience	1996 (Canada)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Oilseed rape	*Brassica napus*	**HCN92 (Topas 19/2)**	ACS-BNØØ7-1	Bayer CropScience	1995 (Canada)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Oilseed rape	*Brassica rapa*	**HCR-1**	not available	Bayer CropScience	1998 (Canada)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Oilseed rape	*Brassica napus*	**MON88302**	MON-883Ø2-9	Monsanto	2012 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Oilseed rape	*Brassica napus*	**MON88302 x MS8 x RF3**	MON-883Ø2-9 x ACS-BNØØ5-8 x ACS-BNØØ3-6	Monsanto	2014 (South Korea)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance , Glyphosate tolerance , Male sterility , Fertility restoration
Oilseed rape	*Brassica napus*	**MON88302 x RF3**	MON-883Ø2-9 x ACS-BNØØ3-6	Monsanto	2014 (South Korea)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance , Glyphosate tolerance , Fertility restoration
Oilseed rape	*Brassica napus*	**MPS961**	not available	BASF Plant Science	1999 (USA)*	0	0	0	0	0	X	0	X	Phytase production
Oilseed rape	*Brassica napus*	**MPS962**	not available	BASF Plant Science	1999 (USA)*	0	0	0	0	0	X	0	X	Phytase production
Oilseed rape	*Brassica napus*	**MPS963**	not available	BASF Plant Science	1999 (USA)	0	0	0	0	0	X	0	X	Phytase production
Oilseed rape	*Brassica napus*	**MPS964**	not available	BASF Plant Science	1999 (USA)*	0	0	0	0	0	X	0	X	Phytase production
Oilseed rape	*Brassica napus*	**MPS965**	not available	BASF Plant Science	1999 (USA)*	0	0	0	0	0	X	0	X	Phytase production
Oilseed rape	*Brassica napus*	**MS1 (B91-4)**	ACS-BNØØ4-7	Bayer CropScience	1995 (Canada)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Male sterility
Oilseed rape	*Brassica napus*	**MS1 x RF1 (PGS1)**	ACS-BNØØ4-7 x ACS-BNØØ1-4	Bayer CropScience	1995 (Canada)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**MS1 x RF2 (PGS2)**	ACS-BNØØ4-7 x ACS-BNØØ2-5	Bayer CropScience	1995 (Canada)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Male sterility, Fertility restoration

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Oilseed rape	*Brassica napus*	**MS1 x RF3**	ACS-BNØØ4-7 x ACS-BNØØ3-6	Bayer CropScience	2006 (China)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**MS8**	ACS-BNØØ5-8	Bayer CropScience	1996 (Canada)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility
Oilseed rape	*Brassica napus*	**MS8 x RF3**	ACS-BNØØ5-8 x ACS-BNØØ3-6	Bayer CropScience	1996 (Canada)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**MS8 x RF3 x GT73 (RT73)**	ACS-BNØØ5-8 x ACS-BNØØ3-6 x MON-ØØØ73-7	Bayer CropScience	2010 (Japan)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**OXY-235**	ACS-BNØ11-5	Bayer CropScience	1997 (Canada)	0	0	X	0	0	0	0	0	Oxynil tolerance
Oilseed rape	*Brassica napus*	**PHY14**	not available	Bayer CropScience	2001 (Japan)*	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**PHY23**	not available	Bayer CropScience	2001 (Japan)*	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**PHY35**	not available	Bayer CropScience	2001 (Japan)*	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**PHY36**	not available	Bayer CropScience	2001 (Japan)*	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Male sterility, Fertility restoration
Oilseed rape	*Brassica napus*	**RF1 (B93-101)**	ACS-BNØØ1-4	Bayer CropScience	1994 (Canada)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Fertility restoration
Oilseed rape	*Brassica napus*	**RF2 (B94-2)**	ACS-BNØØ2-5	Bayer CropScience	1995 (Canada)	0	X	X	0	0	0	0	X	Glufosinate ammonium tolerance, Fertility restoration
Oilseed rape	*Brassica napus*	**RF3**	ACS-BNØØ3-6	Bayer CropScience	1996 (Canada)	0	X	X	0	0	0	0	0	Glufosinate ammonium tolerance, Fertility restoration
Oilseed rape	*Brassica rapa*	**ZSR500**	not available	University of Florida	1997 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Oilseed rape	*Brassica rapa*	**ZSR502**	not available	University of Florida	1997 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Oilseed rape	*Brassica rapa*	**ZSR503**	not available	University of Florida	1997 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Papaya	*Carica papaya*	**55-1**	CUH-CP551-8	Cornell University	1996 (USA)	0	0	0	0	X	0	0	X	Papaya ringspot virus

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
				and University of Hawaii										resistance
Papaya	*Carica papaya*	**63-1**	CUH-CP631-7	Cornell University and University of Hawaii	1996 (USA)	0	0	0	0	X	0	0	X	Papaya ringspot virus resistance
Papaya	*Carica papaya*	**Huanong No. 1**	not available	South China Agricultural University	2006 (China)	0	0	0	0	X	0	0	0	Papaya ringspot virus resistance
Papaya	*Carica papaya*	**X17-2**	UFL-X17CP-6	University of Florida	2008 (USA)	0	0	0	0	X	0	0	X	Papaya ringspot virus resistance
Petunia	*Petunia hybrida*	**Petunia-CHS**	not available	Beijing University	1998 (China)	0	0	0	0	0	X	0	0	Modified Product Quality
Plum	*Prunus domestica*	**C-5**	ARS-PLMC5-6	United States Department of Agriculture - Agricultural Research Service	2007 (USA)	0	0	0	0	X	0	0	X	Plum pox virus resistance
Poplar	*Populus nigra*	**Bt poplar, poplar 12**	not available	Research Institute of Forestry (China)	1998 (China)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Poplar	*Populus sp.*	**Hybrid poplar clone 741**	not available	Research Institute of Forestry (China)	2001 (China)	0	0	0	X	0	0	0	X	Lepidopteran resistance, Multiple insect resistance
Potato	*Solanum tuberosum*	**1210 amk**	not available	Centre Bioengineering, Russian Academy of Sciences	2007 (Russian Federation)*	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**2904/1 kgs**	not available	Centre Bioengineering, Russian Academy of Sciences	2005 (Russian Federation)*	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**AM04-1020**	BPS-A1Ø2Ø-5	BASF Plant Science	2014 (USA)*	0	0	0	0	0	X	0	0	Modified starch/carbohydrate
Potato	*Solanum tuberosum*	**ATBT04-27**	NMK-89367-8	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**ATBT04-30**	NMK-89613-2	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**ATBT04-31**	NMK-8917Ø-9	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**ATBT04-36**	NMK-89279-1	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Potato	*Solanum tuberosum*	**ATBT04-6**	NMK-89761-6	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**BT06**	NMK-89812-3	Monsanto	1994 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**BT10**	NMK-89175-5	Monsanto	1994 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**BT12**	NMK-896Ø1-8	Monsanto	1994 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**BT16**	NMK-89167-6	Monsanto	1994 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**BT17**	NMK-89593-9	Monsanto	1994 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**BT18**	NMK-899Ø6-7	Monsanto	1994 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**BT23**	NMK-89675-1	Monsanto	1994 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**E12**	SPS-ØØE12-8	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**E24**	SPS-ØØE24-2	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**EH92-527-1**	BPS-25271-9	BASF Plant Science	2010 (EU)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate
Potato	*Solanum tuberosum*	**F10**	SPS-ØØF10-7	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**F37**	SPS-ØØF37-7	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum*	**G11**	SPS-ØØG11-9	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Reduced Acrylamide

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
	tuberosum													Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**H37**	SPS-ØØH37-9	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**H50**	SPS-ØØH50-4	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**HLMT15-15**	not available	Monsanto	1998 (USA)*	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Virus Y resistance
Potato	*Solanum tuberosum*	**HLMT15-3**	not available	Monsanto	1998 (USA)*	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Virus Y resistance
Potato	*Solanum tuberosum*	**HLMT15-46**	not available	Monsanto	1998 (USA)*	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Virus Y resistance
Potato	*Solanum tuberosum*	**J3**	SPS-ØØØJ3-4	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**J55**	SPS-ØØJ55-2	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Modified starch/carbohydrate , Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**J78**	SPS-ØØJ78-7	J.R. Simplot Co.	2014 (USA)	0	0	0	0	0	X	0	0	Reduced Acrylamide Potential , Black Spot Bruise Tolerance
Potato	*Solanum tuberosum*	**RBMT15-101**	NMK-89653-6	Monsanto	1997 (USA)	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Virus Y resistance
Potato	*Solanum tuberosum*	**RBMT21-129**	NMK-89684-1	Monsanto	1997 (USA)	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Leaf Roll Virus resistance
Potato	*Solanum tuberosum*	**RBMT21-152**	not available	Monsanto	1998 (USA)*	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Leaf Roll Virus resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Potato	*Solanum tuberosum*	**RBMT21-350**	NMK-89185-6	Monsanto	1997 (USA)	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Leaf Roll Virus resistance
Potato	*Solanum tuberosum*	**RBMT22-082**	NMK-89896-6	Monsanto	1998 (USA)	0	0	X	X	X	0	0	0	Glyphosate tolerance, Coleopteran resistance, Potato Leaf Roll Virus resistance
Potato	*Solanum tuberosum*	**RBMT22-186**	not available	Monsanto	1998 (USA)*	0	0	X	X	X	0	0	0	Glyphosate tolerance, Coleopteran resistance, Potato Leaf Roll Virus resistance
Potato	*Solanum tuberosum*	**RBMT22-238**	not available	Monsanto	1998 (USA)*	0	0	X	X	X	0	0	0	Glyphosate tolerance, Coleopteran resistance, Potato Leaf Roll Virus resistance
Potato	*Solanum tuberosum*	**RBMT22-262**	not available	Monsanto	1998 (USA)*	0	0	X	X	X	0	0	0	Glyphosate tolerance, Coleopteran resistance, Potato Leaf Roll Virus resistance
Potato	*Solanum tuberosum*	**SEMT15-02**	NMK-89935-9	Monsanto	1997 (USA)	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Virus Y resistance
Potato	*Solanum tuberosum*	**SEMT15-07**	not available	Monsanto	1998 (USA)*	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Virus Y resistance
Potato	*Solanum tuberosum*	**SEMT15-15**	NMK-8993Ø-4	Monsanto	1997 (USA)	0	0	0	X	X	0	0	X	Coleopteran resistance, Potato Virus Y resistance
Potato	*Solanum tuberosum*	**SPBT02-5**	NMK-89576-1	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Potato	*Solanum tuberosum*	**SPBT02-7**	NMK-89724-5	Monsanto	1995 (USA)	0	0	0	X	0	0	0	X	Coleopteran resistance
Rice	*Oryza sativa*	**7Crp#10**	not available	National Institute of Agrobiological Sciences (Japan)	2007 (Japan)	0	0	0	0	0	0	X	X	Anti-allergy
Rice	*Oryza sativa*	**GM Shanyou 63**	not available	Huazhong Agricultural University (China)	2009 (China)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Rice	*Oryza sativa*	**Huahui-1/TT51-1**	not available	Huazhong Agricultural University (China)	2009 (China)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Rice	*Oryza sativa*	**LLRICE06**	ACS-OSØØ1-4	Bayer CropScience	1999 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
														tolerance
Rice	*Oryza sativa*	**LLRICE601**	BCS-OSØØ3-7	Bayer CropScience	2006 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Rice	*Oryza sativa*	**LLRICE62**	ACS-OSØØ2-5	Bayer CropScience	1999 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Rice	*Oryza sativa*	**Tarom molaii + cry1Ab**	not available	Agricultural Biotech Research Institute (Iran)	2004 (Iran)	0	0	0	X	0	0	0	X	Lepidopteran resistance
Rose	*Rosa hybrida*	**WKS82/130-4-1**	IFD-524Ø1-4	Suntory Limited (Japan)	2008 (Japan)	0	0	0	0	0	X	0	0	Modified flower color
Rose	*Rosa hybrida*	**WKS92/130-9-1**	IFD-529Ø1-9	Suntory Limited (Japan)	2008 (Japan)	0	0	0	0	0	X	0	0	Modified flower color
Soybean	*Glycine max*	**260-05 (G94-1, G94-19, G168)**	DD-Ø26ØØ5-3	DuPont (Pioneer Hi-Bred)	1997 (USA)	0	0	0	0	0	X	0	X	Modified oil/fatty acid
Soybean	*Glycine max*	**A2704-12**	ACS-GMØØ5-3	Bayer CropScience	1996 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Soybean	*Glycine max*	**A2704-21**	ACS-GMØØ4-2	Bayer CropScience	1996 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Soybean	*Glycine max*	**A5547-127**	ACS-GMØØ6-4	Bayer CropScience	1998 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Soybean	*Glycine max*	**A5547-35**	ACS-GMØØ8-6	Bayer CropScience	1996 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Soybean	*Glycine max*	**CV127**	BPS-CV127-9	BASF Plant Science	2009 (Brazil)	0	0	X	0	0	0	0	0	Sulfonylurea tolerance
Soybean	*Glycine max*	**DAS44406-6**	DAS-444Ø6-6	Dow AgroSciences	2013 (Australia, Canada, New Zealand, South Africa)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, 2,4-D tolerance
Soybean	*Glycine max*	**DAS68416-4**	DAS-68416-4	Dow AgroSciences	2011 (Australia, New Zealand, USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance, 2,4-D tolerance
Soybean	*Glycine max*	**DAS68416-4 x MON89788**	DAS-68416-4 x MON-89788-1	Dow AgroSciences	2013 (Canada)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance, Glyphosate tolerance, 2,4-D tolerance
Soybean	*Glycine max*	**DAS81419**	DAS-81419-2	Dow AgroSciences	2013 (Japan)	0	0	X	X	0	0	0	0	Glufosinate ammonium tolerance, Lepidopteran resistance
Soybean	*Glycine max*	**DP305423**	DP-3Ø5423-1	DuPont (Pioneer Hi-Bred)	2008 (Mexico)	0	0	X	0	0	X	0	0	Sulfonylurea tolerance, Modified oil/fatty acid

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
Soybean	*Glycine max*	**DP305423 x GTS 40-3-2**	DP-3Ø5423-1 x MON-Ø4Ø32-6	DuPont (Pioneer Hi-Bred)	2010 (Japan)	0	0	X	0	0	X	0	0	Glyphosate tolerance, Sulfonylurea tolerance, Modified oil/fatty acid
Soybean	*Glycine max*	**DP356043**	DP-356Ø43-5	DuPont (Pioneer Hi-Bred)	2007 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance, Sulfonylurea tolerance
Soybean	*Glycine max*	**FG72 (FGØ72-2, FGØ72-3)**	MST-FGØ72-3	Bayer CropScience and MS Technologies	2012 (Australia, Canada, Japan, New Zealand, USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance , Isoxaflutole tolerance
Soybean	*Glycine max*	**GTS 40-3-2 (40-3-2)**	MON-Ø4Ø32-6	Monsanto	1995 (Canada)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Soybean	*Glycine max*	**GU262**	ACS-GMØØ3-1	Bayer CropScience	1998 (USA)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance
Soybean	*Glycine max*	**MON87701**	MON-877Ø1-2	Monsanto	2010 (Canada, Mexico, USA)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Soybean	*Glycine max*	**MON87701 x MON89788**	MON-877Ø1-2 x MON-89788-1	Monsanto	2010 (Brazil)	0	0	X	X	0	0	0	0	Glyphosate tolerance, Lepidopteran resistance
Soybean	*Glycine max*	**MON87705**	MON-877Ø5-6	Monsanto	2011 (Australia, Canada, Mexico, New Zealand, USA)	0	0	X	0	0	X	0	0	Glyphosate tolerance, Modified oil/fatty acid
Soybean	*Glycine max*	**MON87705 x MON89788**	MON-877Ø5-6 x MON-89788-1	Monsanto	2012 (Mexico)	0	0	X	0	0	X	0	0	Glyphosate tolerance, Modified oil/fatty acid
Soybean	*Glycine max*	**MON87708**	MON-877Ø8-9	Monsanto	2011 (USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance, Dicamba tolerance
Soybean	*Glycine max*	**MON87708 x MON89788**	MON-877Ø8-9 x MON-89788-1	Monsanto	2010 (Brazil)	0	0	X	0	0	0	0	0	Glyphosate tolerance, Dicamba tolerance
Soybean	*Glycine max*	**MON87712**	MON-87712-4	Monsanto	2013 (USA)	0	X	X	0	0	0	0	0	Glyphosate tolerance, Enhanced Photosynthesis/Yield
Soybean	*Glycine max*	**MON87751**	MON-87751-7	Monsanto	2014 (Canada, Japan, USA)	0	0	0	X	0	0	0	0	Lepidopteran resistance
Soybean	*Glycine max*	**MON87769**	MON-87769-7	Monsanto	2011 (Australia, Canada, New Zealand, USA)	0	0	X	0	0	X	0	0	Glyphosate tolerance, Modified oil/fatty acid
Soybean	*Glycine max*	**MON87769 x MON89788**	MON-87769-7 x MON-89788-1	Monsanto	2012 (Mexico)	0	0	X	0	0	X	0	0	Glyphosate tolerance, Modified oil/fatty acid
Soybean	*Glycine max*	**MON89788**	MON-89788-1	Monsanto	2007 (Canada, Japan, Philippines, Taiwan, USA)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Soybean	*Glycine max*	**SYHTØH2**	SYN-ØØØH2-5	Bayer CropScience	2013 (Russian	0	0	X	0	0	0	0	0	Glufosinate ammonium

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
					Federation)									tolerance, Mesotrione Tolerance
Soybean	*Glycine max*	**W62**	ACS-GMØØ2-9	Bayer CropScience	1996 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Soybean	*Glycine max*	**W98**	ACS-GMØØ1-8	Bayer CropScience	1996 (USA)	0	0	X	0	0	0	0	0	Glufosinate ammonium tolerance
Squash	*Cucurbita pepo*	**CZW3**	SEM-ØCZW3-2	Seminis Vegetable Seeds (Canada) and Monsanto	1994 (USA)	0	0	0	0	X	0	0	X	Cucumber Mosaic Cucumovirus resistance, Zucchini Yellow Mosaic Potyvirus resistance, Watermelon Mosaic Potyvirus 2 resistance
Squash	*Cucurbita pepo*	**ZW20**	SEM-ØZW2Ø-7	Seminis Vegetable Seeds (Canada) and Monsanto	1994 (USA)	0	0	0	0	X	0	0	0	Zucchini Yellow Mosaic Potyvirus resistance, Watermelon Mosaic Potyvirus 2 resistance
Sugar Beet	*Beta vulgaris*	**GTSB77 (T9100152)**	SY-GTSB77-8	Novartis Seeds and Monsanto	1998 (USA)	0	0	X	0	0	0	0	X	Glyphosate tolerance
Sugar Beet	*Beta vulgaris*	**H7-1**	KM-ØØØH71-4	KWS and Monasanto	2003 (Japan)	0	0	X	0	0	0	0	0	Glyphosate tolerance
Sugar Beet	*Beta vulgaris*	**T120-7**	ACS-BVØØ1-3	Bayer CropScience	1998 (USA)	0	0	X	0	0	0	0	X	Glufosinate ammonium tolerance
Sugarcane	*Saccharum sp*	**NXI-1T**	not available	PT Perkebunan Nusantara XI (Persero)	2011 (Indonesia)**	X	0	0	0	0	0	0	X	Drought tolerance
Sugarcane	*Saccharum sp*	**NXI-4T**	not available	PT Perkebunan Nusantara XI (Persero)	2013 (Indonesia)**	X	0	0	0	0	0	0	0	Drought tolerance
Sugarcane	*Saccharum sp*	**NXI-6T**	not available	PT Perkebunan Nusantara XI (Persero)	2013 (Indonesia)**	X	0	0	0	0	0	0	0	Drought tolerance
Sweet pepper	*Capsicum annuum*	**PK-SP01**	not available	Beijing University	1998 (China)	0	0	0	0	X	0	0	0	Cucumber Mosaic Cucumovirus (CMV) resistance
Tobacco	*Nicotiana tabacum*	**C/F/93/08-02**	not available	SEITA S.A. (France)	1994 (EU)	0	0	X	0	0	0	0	0	Oxynil tolerance
Tobacco	*Nicotiana tabacum*	**Vector 21-41**	not available	Vector Tobacco Inc. (USA)	2002 (USA)	0	0	0	0	0	X	0	X	Nicotine reduction
Tomato	*Solanum*	**5345**	not available	Monsanto	1998 (USA)	0	0	0	X	0	0	0	X	Lepidopteran resistance

Crop	Species	Event	OECD code	Developer	First approval	Agronomic properties			Biotic stress resistance		Product specifications		Other traits	
						AS	PB	HT	IR	VR	PQ	PS	MG	Trait(s)
	lycopersicum													
Tomato	*Solanum lycopersicum*	**8338**	CGN-89322-3	Monsanto	1994 (USA)	0	0	0	0	0	X	0	X	Delayed ripening/senescence
Tomato	*Solanum lycopersicum*	**1345-4**	not available	DNA Plant Technology Corporation (USA)	1995 (Canada, USA)	0	0	0	0	0	X	0	X	Delayed ripening/senescence
Tomato	*Solanum lycopersicum*	**35-1-N**	not available	Agritope Inc. (USA)	1996 (USA)	0	0	0	0	0	X	0	X	Delayed ripening/senescence
Tomato	*Solanum lycopersicum*	**B**	SYN-ØØØØB-6	Zeneca Plant Science and Petoseed Company	1994 (USA)	0	0	0	0	0	X	0	X	Delayed fruit softening
Tomato	*Solanum lycopersicum*	**Da**	SYN-ØØØDA-9	Zeneca Plant Science and Petoseed Company	1994 (USA)	0	0	0	0	0	X	0	X	Delayed fruit softening
Tomato	*Solanum lycopersicum*	**Da Dong No 9**	not available	Institute of Microbiology, CAS (China)	1999 (China)	0	0	0	0	0	X	0	0	Modified Product Quality
Tomato	*Solanum lycopersicum*	**F (1401F, h38F, 11013F, 7913F)**	SYN-ØØØØF-1	Zeneca Plant Science and Petoseed Company	1994 (USA)	0	0	0	0	0	X	0	X	Delayed fruit softening
Tomato	*Solanum lycopersicum*	**FLAVR SAVR™**	CGN-89564-2	Monsanto	1992 (USA)	0	0	0	0	0	X	0	X	Delayed fruit softening
Tomato	*Solanum lycopersicum*	**Huafan No 1**	not available	Huazhong Agricultural University (China)	1997 (China)	0	0	0	0	0	X	0	0	Delayed ripening/senescence
Tomato	*Solanum lycopersicum*	**PK-TM8805R (8805R)**	not available	Beijing University	1999 (China)	0	0	0	0	X	0	0	0	Cucumber Mosaic Cucumovirus (CMV) resistance
Wheat	*Triticum aestivum*	**MON71800**	MON-718ØØ-3	Monsanto	2004 (USA)*	0	0	X	0	0	0	0	0	Glyphosate tolerance

Table 3 Reports and EFSA opinions on PMEM for cultivated GM crops

Amylopectin Potato EH92-527-1 Variety Amflora
BASF, 2011, Post-Market Monitoring Report for the Cultivation of Amylopectin Potato EH92-527-1 Variety Amflora in 2010. http://ec.europa.eu/food/plant/gmo/reports_studies/docs/post_market_monitoring_report_en.pdf.
Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from BASF Plant Science Company GmbH on the cultivation of genetically modified potato EH92-527-1 in 2010. EFSA Journal 2012;10(2):2558 [38 pp.]. doi: 10.2903/j.efsa.2012.2558.
BASF, 2012, Post-Market Monitoring Report for the Cultivation of Amylopectin Potato EH92-527-1 Variety Amflora in 2011. http://ec.europa.eu/food/food/biotechnology/docs/post_market_monitoring_report_2011_en.pdf.
Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from BASF Plant Science Company GmbH on the cultivation of genetically modified potato EH92-527-1 in 2011. EFSA Journal 2012;10(12):3015 [35 pp.]. doi: 10.2903/j.efsa.2012.3015
BASF, 2013, Post-Market Monitoring Report for the Cultivation of Amylopectin Potato EH92-527-1 Variety Amflora in 2012. http://ec.europa.eu/food/plant/docs/plant_gmo_report-studies_bps_2012_post-market_monitoring_report_eh92-527-1_en.pdf
Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from BASF Plant Science Company GmbH on genetically modified potato EH92-527-1 in 2012. EFSA Journal 2013;11(10):3445 [10 pp.]. doi: 10.2903/j.efsa.2013.3445
BASF, 2013, Post-Market Monitoring Report for the Cultivation of Amylopectin Potato EH92-527-1 Variety Amflora in 2013. http://ec.europa.eu/food/plant/gmo/reports_studies/docs/amflora_2013_monitoring_report_en.pdf
MON810
Monsanto, 2009, Annual Monitoring Report on the Cultivation of MON 810 in 2008. http://ec.europa.eu/food/plant/docs/plant_gmo_report_studies_revised_report_2013_mon_810_ref_28_en.pdf
Monsanto, 2010, Annual Monitoring Report on the Cultivation of MON 810 in 2009. http://ec.europa.eu/food/plant/gmo/reports_studies/docs/annual_monitoring_report_mon810_2009_en.pdf.
Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON810 in 2009. EFSA Journal 2011;9(10):2376 [66 pp.]. doi: 10.2903/j.efsa.2011.2376
Monsanto, 2011, Annual Monitoring Report on the Cultivation of MON 810 in 2010. http://ec.europa.eu/food/plant/gmo/reports_studies/docs/report_mon_810_en.pdf
Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. EFSA Journal 2012;10(4):2610 [35 pp.]. doi: 10.2903/j.efsa.2012.2610
Monsanto, 2012, Annual Monitoring Report on the Cultivation of MON 810 in 2011. http://ec.europa.eu/food/plant/gmo/reports_studies/docs/report_2012_mon_810_en.pdf.
Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2011. EFSA Journal 2013;11(12):3500 [38 pp.]. doi: 10.2903/j.efsa.2013.3500
Monsanto, 2013, Annual Monitoring Report on the Cultivation of MON 810 in 2012. http://ec.europa.eu/food/plant/gmo/reports_studies/docs/report_2012_mon_810/report_2012_mon_810_en.pdf.
Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2012. EFSA Journal 2014;12(6):3704, 29 pp. doi:10.2903/j.efsa.2014.3704

Monsanto, 2014, Annual Monitoring Report on the Cultivation of MON 810 in 2013.
http://ec.europa.eu/food/plant/docs/plant_gmo_report_studies_report_2013_mon_810_en.pdf.

Scientific Opinion on the annual post-market environmental monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2013. EFSA Journal 2015;13(3):4039 [11 pp.]. doi: 10.2903/j.efsa.2015.4039

Monsanto, 2015, Revised Annual Monitoring Report on the cultivation of MON 810 in the 2013 growing season
http://ec.europa.eu/food/plant/docs/plant_gmo_report_studies_report_2013_mon_810_revised_en.pdf

Scientific opinion on the revised annual post-market environmental monitoring (PMEM) report on the cultivation of genetically modified maize MON 810 in 2013 from Monsanto Europe S.A. EFSA Journal 2015;13(11):4295, 37 pp. doi:10.2903/j.efsa.2015.4295

Annex 6 Research related to gene therapy

Table 1 Studies commissioned by authorities in the Netherlands relative to ERA for gene therapy

the Netherlands Ministry of Environment
Environmental risk assessment of replication competent viral vectors in gene therapy trials (Report 601850001/2008) (Van de Akker, 2013) Environmental risk assessment of replication competent viral vectors in gene therapy trials: potential effects of inserted sequences (Van de Akker *et al*., 2013) Guidance document with one example applying the proposed ERA template. The accompanying publication builds on the report and stresses the importance of the characteristics of the insert.
COGEM studies
Recombinant and chimeric viruses: Evaluation of risks associated with changes in tropism (CGM 2005-04) Combining virus parts may result in viruses with poorly understood characteristics. A desk study was performed to gain a better understanding of the risks associated with changes in the tropism of recombinant or chimeric viruses.
Inventarisatie van sheddingdata en analyses: mogelijkheden voor standaardisering (CGM 2006-04) Schenk-Braat E.A., van Mierlo M.M., Wagemaker G., Bangma C.H., Kaptein L.C. (2007) An inventory of shedding data from clinical gene therapy trials. J Gene Med. 9(10): 910-21. The study provides a broad inventory of of published shedding data from historical clinical trials to support evidence-based risk assessment. To overcome the lack of harmonisation between the tests a uniform guidance is presented.
Gene therapy with naked DNA: Potential steps towards deregulation (CGM 2010-06) Naked DNA may pose a risk for the creation and spreading of new GMOs via the uptake by somatic cells, germ line cells, viruses or bacteria. Although considered as a very low risk, for use in gene therapy the effect of improved expression vectors, synthetic carrier systems and physical and mechanical delivery techniques is assessed.
International Medical Tourism from the Netherlands for gene therapy (CGM 2010-07) The study aimed to give an indication on the extent of medical tourism, especially for gene therapy and found that the number of persons travelling abroad for this type of medical treatment is very low. Very little data related to the type of treatment, let alone shedding is available.
Replication-competent non-human viruses for use in clinical gene therapy: an inventory study (CGM 2010-10) The study inventoried the use of non-human viruses in the light of the risk of virus adaptation of non-human viruses to humans and the associated risk of the virus adapting to increase replication in humans and spread into the environment. Based on the available data, each of the viruses was assigned to one of 5 relative environmental risk categories.
Gene therapy in China. From a Dutch perspective (CGM 2011-03) The rapidly evolving developments in the gene therapy field in China are reported on. With increasing numbers of clinical studies mostly targeted to treat cancer and therapies approved, the country becomes attractive for medical tourism. Publication of results, especially on environmental risks, is poor.
Methodology for environmental risk assessments in medical and veterinary biotechnology (CGM 2012-04) In this report the COGEM approach in conduting ERA was analysed based on the large number of ERAs relating to human and veterinary medicine research and development COGEM has delivered, both for contained use as well as deliberate release.
Gene therapy clinical trials: what about the environment? A comparison between the Netherlands and North America (CGM/2012-07) The report compares how the aspect of potential environmental risk is addressed in assessing clinical trials between the Netherlands, Canada and the USA. While in the

Netherlands this is evaluated from the first phase trials, in the USA and Canada only in specific cases a full assessment is necessary for early clinical trials. Only when nearing market introduction a full ERA is required.

GM Vaccines: From bench to bedside (CGM 2014-08)

An inventory is made of GM vaccines currently in the market and in the development pipeline for human and veterinary use.

COGEM events

Bleijs D.A., 2005, Workshop on shedding data from gene therapy studies with viral vectors. Amsterdam, January 22, 2004. J Gene Med 7, 256–259.

The publication summarises the presentations on adeno-associated virus vectors, canarypox virus (ALVAC), adenoviral vectors, retroviral vectors.

Table 2 Research projects sponsored by the EU relative to ERA for gene therapy

The following information is retrieved from EC, 2001, EC, 2010 and the Cordis website[98]

FP1 – BAP Biodiversity Action Plan research programme (EEC) (1984-1988)
Monitoring of potential risk linked to the use of modified live viruses for antirabies vaccination of foxes (BAP-0368/0381/0382)(1989-1990)
The safety and efficacy is tested of a recombinant vaccinia virus expressing the immunogenic G protein of rabies virus that was released in the field. The monitoring found a recombination event between the recombinant virus and a wild orthopoxvirus. Subsequent research showed that the perceived risk was negligible.
FP2 - BRIDGE Specific research and technological development programme (EEC) in the field of biotechnology (1987-1991)
Safety of genetically engineered retroviruses used for gene transfer (BIOT-CT91-0286)(1991-1993)
Basic research studying the risk of generation of recombinant viruses and of transfer sequences to the target cell, specificity of gene delivery, safer packaging systems.
Assessment of environmental impact from the use of live recombinant virus vaccines (BIOT-CT91-0289)(1991-1993)
Vaccinia virus for wildlife vaccination.
FP3 (1990-1994)
FP4 (1994-1998)
Biosafety of vaccines based on self-replicating recombinant alphavirus (BIO4-CT98-0031)(1998-2000)
Semliki Forest virus vector is used to prepare vaccines against influenza, *louping ill* flavivirus and *infectious bursal disease* virus. Experiments to study vaccine and cytokine persistence show that persistence is less than 7 days.
Genetic and immunological safety of DNA vaccines (BIO4-CT96-0637)(1996-1999)
Plasmid DNA vaccines are studied for undesirable immune responses and assayed to assess integration of plasmid DNA. The DNA was directed towards influenza and *respiratory syncytial virus*. The purity of the plasmid preparation affects inflammatory response, but no integration was detected.
Biosafety of mucosa-specific RNA-vectors expressing foreign antigens and recombinant antibodies for prevention of disease (BIO4-CT98-0239)(1998-2000)
The objective is to test stability and biosafety of *Porcine Coronavirus*-derived expression system for vaccine development and gene therapy. Two human-specific versions are made. A recombinant swine model was used to test biosafety.
FP5 (1998-2002)
FP6 (2002-2006)
FLUVACC, Live attenuated replication-defective influenza vaccine (LSH-2004-1.2.5-3)(2005-2010)
The project is to develop an intranasal vaccine against pandemic influenza, based on reverse genetics. This technology will permit to rapidly generate replication-defective strains from new emerging influenza viruses that are safe and effective.
HEPACIVAC, New preventative and therapeutic Hepatits C vaccines: from pre-clinical to phase I (LSH-2005-1.2.4-2)(2007-20012)
A gene based HCV vaccine candidate using adenoviral vectors for delivery is investigated.

[98] http://cordis.europa.eu/projects/home_en.html

Chiron vaccine candidate consists in recombinant HCV glycoproteins, gpE1 and gpE2 associated to resemble to a pre-virion envelope structure.

CLINIGENE, European network for the advancement of clinical gene transfer and therapy (LSH-2004-1.2.4-3)(2006-2011)[99]

The project is to define precise quality and safety standards for clinical gene transfer. Activities are planned serving integration towards the generation of reference/standard profile data-bases on AAV, gamma-retrovirus, lentivirus, adenovirus, genetically-modified cells & non-viral vectors.

CONSERT, Concerted Safety and Efficiency Evaluation of Retroviral Transgenesis for Gene Therapy of Inherited Diseases (LSH-2003-1.2.4-1 and LSH-2003-1.2.4-2)(2004-2009).

A safety and efficiency evaluation of genetic stem cell modification using retroviral vector-mediated transgenesis: lenti-, spuma- and gamma-retroviral vectors. Safety is studied in preclinical disease models.

EPI-VECTOR, Episomal vectors as gene delivery systems for therapeutic application (LSH-2003-1.2.4-7)(2004-2008)

Extra-chromosomal gene expression vectors are developed to avoid integration in the genome.

GIANT, Gene therapy: an integrated approach for neoplastic treatment (LSH-2003-1.2.4-1)(2005-2010)

Vectors are developed for better targeting and to avoid vector immunogenicity (stealthing) to reduce bloodstream and immune-mediated reduction of effective vector concentration. Clinical testing is planned.

DENDRITOPHAGES, Therapeutic cancer vaccines (LSH-2002-1.2.4-6)(2004-2007)

Vectors are developed on the basis of porcine parvovirus-like particles which were shown to target dendritic cells very efficiently and specifically, allowing highly efficient presentation of delivered antigens to T cells. Safety and efficacy Phase I/II human clinical are envisioned.

THERADPOX, Optimised and novel oncolytic adenoviruses and pox viruses in the treatment of cancer: Virotherapy combined with molecular chemotherapy (LSH-2004-1.2.4-7)(2005-2008)

The project is to improve the safety and therapeutic efficacy of oncolytic viruses.

THOVLEN, Targeted Herpesvirus-derived Oncolytic Vectors for Liver cancer European Network (LSH-2004-1.2.4-7)(2006-2009)

The objective it to develop safe and efficient herpes simplex virus type 1 (HSV-1)-derived oncolytic vectors, designed to strictly target and eradicate human hepatocellular carcinomas.

FP7
2007-2013

GENEGRAFT, Phase I/II ex vivo gene therapy clinical trial for recessive dystrophic epidermolysis bullosa using skin equivalent grafts genetically corrected with a COL7A1-encoding SIN retroviral vector (HEALTH.2010.2.4.4-1)(2011-2016)

The aim is to treat RDEB patients by *ex vivo* gene therapy using autologous skin grafts made of primary keratinocytes and fibroblasts genetically corrected with a safe (SIN) retroviral vector expressing type VII collagen under the control of the EF1alpha promoter.

AIPGENE, Augmenting PBGD expression in the liver as a Novel Gene therapy for Acute Intermittent Porphyria (HEALTH.2010.2.4.4-1)(2011-2014)

A phase I clinical trial was performed using the AAV5-AAT-PBGD, an adeno-associated, replication-incompetent virus that delivers the porphobilinogen deaminase (PBGD) gene directly into liver cells.

[99] http://www.clinigene.eu/

Annex 7 Research related to application dossiers for clinical trials on gene therapy

Table 2 Clinical trials on gene therapy in the EU

Source: JRC's Deliberate Release and Placing on the EU Market of GMOs - GMO Register

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/BE/15/BVW1	Amgen Limited	2015	2016-2023	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	Ib/III
B/DE/12/1750	apceth GmbH & Co.KG	2012	2013-2015	virus	Retrovirus	*Murine leukemia virus*	herpes simplex virus thymidine kinase; puromycin resistance gene	Cancer	Gastrointestinal Cancer	I/II
B/DE/14/2247	Universitätsklinikum Hamburg-Eppendorf	2014	2014	virus	Vesiculo virus	*Vesicular stomatitis virus*	Zaire Ebola transmembrane glycoprotein gene	Infecteous disease	Ebola	I
B/DE/14/PEI/2133	Amgen Limited	2014	2014-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	Ib/II
B/DE/14/PEI/2170	uniQure Biopharma B.V.	2014	2014-2015	virus	Dependovirus	*Adeno-associated virus*	human factor IX gene	Monogenic disease	Haemophilia B	
B/DE/14/PEI/2194	Amgen Limited	2014	2014-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	II
B/DE/15/PEI/2260	Transgene S.A.	2015	2014-2017	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	HBV fusion protein Core-Pol-Env	Infecteous disease	Hepatitis B infection	I/IB
B/ES/15/07	Amgen Limited	2015	2015-2017	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	I
B/NL/15/002	University Medical Center Utrecht	2015	2015-2020	plasmid DNA	n.a.	n.a.	Human Hepatocyte Growth Factor gene; kanamycin resistance gene	Cardiovascular disease	Critical limb ischemia	III
B/NL/15/003	Stichting Het Nederlands Kanker Instituut	2015	2015-2025	plasmid DNA	n.a.	n.a.	Human papilloma virus (HPV) E6 or E7 oncoprotein gene; kanamycin resistance gene	Cancer	Cancers of the cervix, vulva, vagina, penis, oropharynx and anus.	
B/NL/15/008	Academisch Ziekenhuis Maastricht	2015	2015-2019	plasmid DNA	n.a.	n.a.	Human Hepatocyte Growth Factor gene; kanamycin resistance gene	Cardiovascular disease	Critical limb ischemia	III

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/NL/14/006	uniQure Biopharma B.V.	2014	2014-2015	virus	Dependovirus	*Adeno-associated virus*	human factor IX gene	Monogenic disease	Haemophilia B	
B/NL/14/007	uniQure Biopharma B.V.	2014	2014-2015	virus	Dependovirus	*Adeno-associated virus*	human factor IX gene	Monogenic disease	Haemophilia B	
B/NL/14/008	uniQure Biopharma B.V.	2014	2014-2015	virus	Dependovirus	*Adeno-associated virus*	human factor IX gene	Monogenic disease	Haemophilia B	
B/NL/15/001	uniQure Biopharma B.V.	2015	2014-2015	virus	Dependovirus	*Adeno-associated virus*	human factor IX gene	Monogenic disease	Haemophilia B	
B/HU/15/01	Transgene S.A.	2015	2011-2019	virus	Orthopoxvirus	*Vaccinia virus*	human MUC1 protein and for the human IL2 genes	Cancer	small cell lung cancer	IIb/III
B/FR/15/GT05	GENETHON	2015	2015-2019	virus	Lentivirus	*Human immunodeficiency virus (HIV)*	human CYBB gene	Monogenic disease	X-Linked Chronic Granulomatous Disease	I/II
B/FR/15/GT06	Amgen Limited	2015	2015-2017	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	II
B/FR/15/GT07	Amgen Limited	2015	2015-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	II
B/ES/15/04	Amgen Limited	2015	2015-2017	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	IIIb
B/FR/15/GT03	Institut Bergonié	2015	2014-2017	virus	Orthopoxvirus	*Vaccinia virus*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF); b-galactosidase gene	Cancer	breast cancer and soft tissue sarcoma	Ib/II
B/FR/15/GT04	Bluebird bio, Inc	2015	2013-2015	virus	Lentivirus	*Human immunodeficiency virus-type 1*	human ATP-binding cassette, sub-family D, member 1 (ABCD1) gene	Monogenic disease	childhood cerebral adrenoleukodystrophy	II/III
B/FR/15/GT01	Amgen Limited	2015	2014-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	Ib/II
B/FR/15/GT02	Transgene S.A.	2015	2014-2017	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	HBV fusion protein Core-Pol-Env gene	Infecteous disease	Hepatitis B infection	I/IB
B/GB/15/R47/01/NI	Prokarium Ltd.	2015	2015-2018	bacterium	Salmonella	*enterica*	synthetic gene encoding the ETEC vaccine protein CF10LTBSTp	Infecteous disease	Typhoid Fever and Enterotoxigenic Escherichia Coli	I

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/SE/15/EU-2013-005579-42	uniQure Biopharma B.V.	2015	2015	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	human factor IX gene	Monogenic disease	Haemophilia B	
B/HU/14/01	Amgen Limited	2014	2014-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	II
B/ES/14/08	Amgen Limited	2014	2015-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	II
B/ES/14/09	Laboratorios del Dr. Esteve, S.A.	2014	2015-2016	virus	Dependovirus	*Adeno-associated Virus*	human Sulfamidase gene	Monogenic disease	Mucolopolysaccharidosis IIIA	I/II
B/BE/14/BVW1	Amgen Limited	2014	2014-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	II
B/ES/14/05	Amgen Limited	2014	2014-2016	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	II
B/ES/14/06	Amgen Limited	2014	2014-2019	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	Ib/II
B/SE/14/EU-2014-000185-22	Amgen Limited	2014	2014-2019	virus	Simplexvirus	*Herpes simplex virus 1*	human granulocyte macrophage colony-stimulating factor gene (hGM-CSF)	Cancer	Melanoma	Ib/II
B/IE/14/01	St. James's Hospital	2014	2014-2015	virus	Mastadenovirus	*chimpanzee adenovirus type 3*	NSmut gene	Infecteous disease	AIDS	I
B/IE/14/02	St. James's Hospital	2014	2014-2015	virus	Orthopoxvirus	*Vaccinia virus*	NSmut gene	Infecteous disease	AIDS	I
B/DE/13/PEI/1863	GENETHON	2013	2013-2018	virus	Lentivirus	*Human immunodeficiency virus-type 1*	human CYBB gene (GP91-PHOX gene)	Monogenic disease	X-linked chronic granulomatous disease	I/II
B/FR/13/GT06	Astellas Pharma	2013	2013-2016	plasmid DNA	n.a.	*n.a.*	hCMV glycoprotein B or hCMV phosphoprotein 65 (pp65); kanamycin resistance gene	Infecteous disease	cytomegalovirus infection	III
B/FR/13/GT04	Oxford BioMedica UK Ltd	2013	2013-2015	virus	Lentivirus	*Equine Infectious Anaemia Virus*	myosin (motor) protein VIIA gene	Monogenic disease	Retinitis Pigmentosa Associated with Usher Syndrome Type 1B	I/IIa

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/FR/13/GT05	GENETHON	2013	2013-2014	virus	Dependovirus	*Adeno-associated virus*	human NADH Dehydrogenase 4 gene	Monogenic disease	Leber Hereditary Optic Neuropathy	I/IIa
B/FR/13/GT03	Institut national de la santé et de la recherche médicale	2013	2013-2015	plasmid DNA	n.a.	n.a.	six proteins or protein fragments of HIV (three regulatory gene products Rev, Nef, Tat and structural gene products (Pol, Gag, Env)	Infecteous disease	AIDS	I/II
B/HU/13/01	Celladon Corporation	2013	2013	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/DE/13/PEI/1827	BN ImmunoTherapeutics, Inc.	2013	2012-2013	virus	Orthopoxvirus	*Vaccinia virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/NL/12/004	University Medical Center Groningen	2012	2013-2015	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/NL/12/006	Academic Medical Center Amsterdam	2012	2013-2015	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/FR/13/GT01	Assistance Publique Hôpitaux de Paris	2013	2013-2016	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	pilot
B/FR/13/GT02	Institut Pasteur	2013	2013	virus	Dependovirus	*Adeno-associated virus*	human alpha-N-acetylglucosaminidase gene	Monogenic disease	Mucolopolysaccharidosis IIIA	I/II
B/NL/12/005	University Medical Center Utrecht	2012	2013-2014	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/NL/13/002	Stichting Het Nederlands Kanker Instituut	2013	2013-2023	plasmid DNA	n.a.	n.a.	fusion protein domain1 of tetanus toxin fragment C and the shuffled version of the Human papilloma virus (HPV) E7 oncoprotein	Cancer	squamous cell cancer	
B/ES/13/18	Clínica Universidad de Navarra	2013	2013-2015	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	integrin-binding RGD-4C peptide sequence	Cancer	glioblastoma	I
B/IE/13/01	Children's Hospital of Philadelphia	2013	2013-2015	virus	Dependovirus	*Adeno-associated virus*	human Factor IX gene	Monogenic disease	Haemophilia B	I
B/GB/13/R46/01	Celladon Corporation	2013	2013-2015	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/GB/13/R46/01/S	Celladon Corporation	2013	2013-2015	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/DE/12/PEI1713	JW Goethe University	2012	2013-2015	virus	Retrovirus	*Murine leukemia virus*	NADPH oxidase 2 (CYBB or gp91phox) gene	Monogenic disease	Chronic granulomatous disease	I/II
B/DE/12/PEI1667	Celladon Corporation	2012	2012-2014	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/DE/10/PEI1279	MolMed SpA	2010	2012-2015	virus	Retrovirus	*Murine leukemia-related retrovirus*	Herpes Simplex Virus Thymidine Kinase (HSV-tk Mut 2) and truncated Low Nerve Growth Factor Receptor (ΔLNGFR) genes	Cancer	Leukemia	III
B/DE/11/PEI1393	VAXIMM GmbH	2011	2011-2012	bacterium	Salmonella	*Salmonella enterica serovar typhi*	human vascular endothelial growth factor receptor 2.	Cancer	Pancreatic cancer	I
B/ES/13/04	VCN Biosciences S.L.	2013	2012-2014	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	matrix-degrading enzyme (hyaluronidase) gene	Cancer	solid tumours	I
B/ES/13/05	VCN Biosciences S.L.	2013	2012-2015	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	matrix-degrading enzyme (hyaluronidase) gene	Cancer	Pancreatic cancer	I
B/BE/12/BVW2	Celladon Corporation	2012	2012-2014	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/DE/11/PEI1419	Transgene S.A.	2011	2011-2015	virus	Orthopoxvirus	*Vaccinia virus*	human mucine 1 (MUC1) and the human interleukin-2 (IL2) gene	Cancer	small cell lung cancer	IIb/III
B/DE/11/PEI1491	Transgene S.A.	2011	2012-2013	virus	Orthopoxvirus	*Vaccinia virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Hepatocellular Carcinoma	IIb
B/DE/11/PEI1332	Transgene S.A.	2011	2011-2012	virus	Orthopoxvirus	*Vaccinia virus*	Hepatitis C virus (HCV) non structural proteins NS3, NS4 and NS5B	Infecteous disease	Hepatitis C	I
B/FR/12/GT06	Transgene S.A.	2012	2013	virus	Orthopoxvirus	*Vaccinia virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Colorectal Carcinoma	I/IIa
B/SE/12/EU-2012-001700-37	Celladon Corporation	2012	2012-2014	virus	Dependovirus	*Adeno-associated virus*	sarcoplasmic reticulum calcium ATPase 2a (hSERCA2a)	Cardiovascular disease	Heart Failure	IIb
B/NL/12/002	Erasmus MC, Rotterdam	2012	2012-2022	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	Human serotype 2 adenovirus E1A (Ad2 E1A) gene	Cancer	Prostate Cancer	

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/GB/12/R44/01/NI	BN ImmunoTherapeutics, Inc.	2012	2012-2013	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/FR/12/GT05	Transgene S.A.	2012	2012-2014	virus	Orthopoxvirus	*Vaccinia virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Hepatocellular Carcinoma	II
B/ES/12/37	Centro de Investigaciones Energéticas Medioambientales y Tecnológicas	2012	2013-2016	virus	Lentivirus	*Human immunodeficiency virus-type 1*	FANCA gene	Monogenic disease	Fanconi anemia subtype A	I/II
B/FR/12/GT04	Bluebird bio, Inc	2012	2013-2016	virus	Lentivirus	*Human immunodeficiency virus-type 1*	βA-T87Q-globin gene	Monogenic disease	Sickle Cell Anemia and β-Thalassemia Major	I/II
B/GB/12/R44/01/S	BN ImmunoTherapeutics, Inc.	2012	2012-2013	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/GB/12/R45/01	International AIDS Vaccine Initiative, New York	2012	2012-2015	virus	Respirovirus	*Sendai virus*	synthetic HIV Gag gene	Infecteous disease	AIDS	I
B/GB/12/R44/01	BN ImmunoTherapeutics, Inc.	2012	2012-2013	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/FR/12/GT03	Inserm	2012	2012-2017	virus	Dependovirus	*Adeno-associated Virus*	Human Arylsulfatase A (ARSA) gene	Monogenic disease	Metachromatic Leukodystrophy	I/II
B/ES/12/40	Transgene S.A.	2012	2012-2014	virus	Orthopoxvirus	*Vaccinia virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Hepatocellular Carcinoma	II
B/NL/12/001	Erasmus MC	2012	2012-2020	virus	Orthopoxvirus	*Vaccinia virus*	influenza virus hemagglutinin gene	Infecteous disease	Influenza	
B/ES/12/39	Transgene S.A.	2012	2012-2013	virus	Orthopoxvirus	*Vaccinia virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Colorectal Carcinoma	I/IIa

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/ES/12/35	Digna Biotech SL	2012	2012-2013	virus	Dependovirus	*Adeno-associated virus*	human porphobilinogen deaminase (cohPBGD)	Monogenic disease	Acute Intermittent Porphyria	I
B/FR/12/GT02	THERAVECTYS	2012	2012-2013	virus	Lentivirus	*Human immunodeficiency virus-type 1*	sequence encoding clustered peptides and epitopes of the HIV-1 clade B, Gag, Pol and Nef proteins	Infecteous disease	AIDS	I/II
B/FR/12/GT01	BN ImmunoTherapeutics, Inc.	2012	2012	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/BE/12/BVW1	THERAVECTYS	2012	2012-2013	virus	Lentivirus	*Human immunodeficiency virus-type 1*	sequence encoding clustered peptides and epitopes of the HIV-1 clade B, Gag, Pol and Nef proteins	Infecteous disease	AIDS	I/II
B/BE/11/BVW2	BN ImmunoTherapeutics, Inc.	2012	2012	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/NL/11/002	University Medical Center Groningen	2011	2012-2022	virus	Alphavirus	*Semliki Forest virus*	human papillomavirus antigens	Cancer	Cancers of the cervix, vulva, vagina, penis, oropharynx and anus.	
B/IS/12/01	BN ImmunoTherapeutics, Inc.	2012	2012	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/EE/12/01	BN ImmunoTherapeutics, Inc.	2012	2012	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/ES/12/09	IrsiCaixa AIDS Research Institute	2012	2012	virus	Mastadenovirus	*chimpanzee adenovirus type 63*	sequence for the HIV-1 specific T-cell immunogen	Infecteous disease	AIDS	
B/ES/12/10	IrsiCaixa AIDS Research Institute	2012	2012	virus	Mastadenovirus	*chimpanzee adenovirus type 63*	sequence for the HIV-1 specific T-cell immunogen	Infecteous disease	AIDS	
B/ES/12/11	Transgene S.A.	2012	2012-2013	virus	Orthopoxvirus	*Vaccinia virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Hepatocellular Carcinoma	IIb

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/ES/12/14	BN ImmunoTherapeutics, Inc.	2012	2012	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/ES/12/17	FUNDACION PARA LA INVESTIGACION BIOMEDICA HOSPITAL UNIVERSITARIO INFANTIL NIÑO JESUS	2012	2011-2012	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	modifications to restrict adenovirus replication to tumor cells (with high free E2F-1 levels)	Cancer	solid tumors	I
B/CZ/12/01	BN ImmunoTherapeutics, Inc.	2012	2012-2014	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/GB/11/R44/01	BN ImmunoTherapeutics, Inc.	2011	2012	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/GB/11/R44/01/W	BN ImmunoTherapeutics, Inc.	2011	2011-2012	virus	Orthopoxvirus; Avipox virus	*Vaccinia virus; Fowlpox virus*	human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules	Cancer	Prostate Cancer	III
B/BE/11/BVW1	Transgene S.A.	2011	2011-2015	virus	Orthopoxvirus	*Vaccinia virus*	human mucine 1 (MUC1) and the human interleukin-2 (IL2)	Cancer	non-small cell lung cancer	IIb/III
B/DE/08/PEI706	MedImmune, LLC	2008	2009-2012	virus	Respirovirus	*Bovine Parainfluenza Virus Type 3*	hPIV3 fusion protein (F) and hemagglutinin-neuraminidase (HN)	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3	I/IIa
B/HU/11/01	Transgene S.A.	2011	2011-2015	virus	Orthopoxvirus	*Vaccinia virus*	human mucine 1 (MUC1) and the human interleukin-2 (IL2)	Cancer	non-small cell lung cancer	IIb/III
B/DE/09/PEI984	Mologen AG	2009	2010-2015	plasmid DNA	n.a.	n.a.	GM-CSF, IL-7, CD80 and CD154 genes	Cancer	Renal Cell Carcinoma	I/II
B/DE/10/PEI1035	Transgene S.A.	2010	2010-2013	virus	Orthopoxvirus	*Vaccinia virus*	Hepatitis C virus (HCV) non structural proteins NS3, NS4 and NS5B genes	Infecteous disease	chronic genotype 1 hepatitis C	II

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/DE/10/PEI1173	Genelux GmbH	2010	2011-2013	virus	Orthopoxvirus	*Vaccinia virus*	sea pansy Renilla reniformis luciferase and humanized Aequorea victoria green fluorescent protein fusion gene; E. coli beta-galactosidase gene; E. coli beta-D-glucuronidase gene; human transferrin receptor sequence	Cancer	peritoneal carcinomatosis	I/II
B/DE/05/PEI35	Hesperion Ltd	2005	2005-2006	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	human vascular endothelial growth factor (VEGF121)	Cardiovascular disease	angina	
B/DE/06/PEI210	Bavarian Nordic GmbH	2006	2006-2007	virus	Orthopoxvirus	*Vaccinia virus*	synthetic DNA fragment encoding 21 conserved CTL epitopes derived from structural and regulatory/accessory proteins of HIV-1 (Gag, Pol, Vpr, Nef, Rev, and Env); synthetic universal HTL epitope PADRE®; synthetic DNA fragment encoding 18 HTL epitopes derived from structural and regulatory/accessory proteins of HIV-1 (Gag, Pol, Vpr, Nef, Rev, and Env)	Infecteous disease	AIDS	I
B/DE/06/PEI231	Transgene S.A.	2006	2005-2008	virus	Orthopoxvirus	*Vaccinia virus*	human mucine 1 (MUC1) and the human interleukin-2 (IL2)	Cancer	non-small cell lung cancer	IIb
B/DE/08/PEI574	Vakzine Projekt Management GmbH	2008	2008-2009	bacterium	Mycobacterium	*Mycobacterium bovis*	Listeriolysin (hly) gene	Infecteous disease	tuberculosis	I
B/NL/11/001	Netherlands Cancer Institute	2011	2011-2021	virus	Gammaretrovirus	*Moloney murine leukemia virus*	Mart-1 specific T cell receptor (beta and alpha chain) gene	Cancer	Melanoma	
B/NL/11/003	Leiden University Medical Center	2011	2012-2017	virus	Gammaretrovirus	*Moloney murine leukemia virus*	T-cell receptor (TCR) beta chain linked with a 2A signal peptide to TCR alpha chain.	Cancer	hematological malignancies	
B/IE/11/451-A	Royal College of Surgeons In Ireland	2011	2011	virus	Mastadenovirus	*chimpanzee adenovirus type 63*	Plasmodium falciparum circumsporozoite protein gene	Infecteous disease	Malaria	I
B/IE/11/451-A	Royal College of Surgeons In Ireland	2011	2011-2012	virus	Mastadenovirus	*chimpanzee adenovirus type 63*	Plasmodium falciparum circumsporozoite protein gene	Infecteous disease	Malaria	I

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/ES/11/28	Transgene S.A.	2011	2011-2015	virus	Orthopoxvirus	*Vaccinia virus*	human mucine 1 (MUC1) and the human interleukin-2 (IL2)	Cancer	non-small cell lung cancer	IIb/III
B/ES/11/27	FUNDACION PARA LA INVESTIGACION BIOMEDICA HOSPITAL UNIVERSITARIO INFANTIL NIÑO JESUS	2011	2011-2012	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	modifications to restrict adenovirus replication to tumor cells (with high free E2F-1 levels)	Cancer	tumors of the Central Nervous System	I
B/ES/11/25	Clínica Universidad de Navarra	2011	2011-2013	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	hepatitis C virus (HCV) NS3 protein gene	Infecteous disease	hepatitis C	I/II
B/SE/10/EU-2010-023103-94	Uppsala University	2010	2011-2013	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	human CD40L (CD154) gene	Cancer	Melanoma	I/IIa
B/GB/10/R40/01	Emergent Product Development UK Ltd	2010	2011-2014	bacterium	Salmonella	*Salmonella enterica serovar typhi*	two deletion mutations	Infecteous disease	typhoid fever	
B/NL/10/002	Utrecht University	2010	2011-2015	plasmid DNA	n.a.	n.a.	human Tyrosinase gene	Cancer	Melanoma	
B/RO/10/08	Parexel International Romania SRL	2009	2010-2013	virus	Orthopoxvirus	*Vaccinia virus*	Hepatitis C virus (HCV) non structural proteins NS3, NS4 and NS5B genes	Infecteous disease	hepatitis C	II
B/IE/10/362	TMC Pharma Services Ltd	2010	2010-2011	virus	Dependovirus	*Adeno-associated virus*	Alpha 1 Antitrypsin gene	Monogenic disease	Alpha 1 Antitrypsin Deficiency	II
B/NL/10/003	Leiden University Medical Center	2010	2010-2020	plasmid DNA	n.a.	n.a.	Fusion protein domain1 of tetanus toxin fragment C and MART-1(aa 26-35)	Cancer	Melanoma	
B/SE/10/EU-2010-019936-11	Swedish institute for Infectious Disease ControlInserm Karolinska University Hospital	2010	2010-2011	bacterium	Bordetella	*Bordetella pertussis*	altered pertussis toxin (PTX) and tracheal cytotoxin (TCT) gene and deletion of dermonecrotic toxin (DNT) gene	Infecteous disease	pertussis	I
B/ES/10/42	Transgene S.A.	2010	2010-2013	virus	Orthopoxvirus	*Vaccinia virus*	Hepatitis C virus (HCV) non structural proteins NS3, NS4 and NS5B genes	Infecteous disease	hepatitis C	
B/NL/08/008	Erasmus MC	2008	2010-2013	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	short sequence encoding an integrin-binding RGD-4C peptide	Cancer	Glioblastoma Multiforme	I/II
B/NL/08/009	Glioblastoma Multiforme	2008	2010-2013	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	short sequence encoding an integrin-binding RGD-4C peptide	Cancer	Glioblastoma Multiforme	I/II

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/FI/09/1MA	MedImmune, LLC	2009	2010	virus	Influenzavirus A and B	*Influenza A and B*	6:2 genetic reassortants	Infecteous disease	Influenza	
B/ES/09/64	Institut Català d'Oncologia - IDIBELL	2009	2010-2011	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	modifications to restrict adenovirus replication to tumor cells (with high free E2F-1 levels)	Cancer	Melanoma	I
B/SE/09/EU-2009-011478-13	Swedish institute for Infectious Disease Control	2009	2009-2012	plasmid DNA	n.a.	n.a.	multiple HIV-1 genes (a.o. truncated envelope gene from HIV-1)	Infecteous disease	AIDS	I
B/GB/09/R43/01	MedImmune, LLC	2009	2009-2012	virus	Respirovirus	*Bovine Parainfluenza virus type 3*	Human Parainfluenza virus type 3 (hPIV3) F and HN genes; Respiratory Syncytial virus (RSV) F gene	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3	I/IIa
B/ES/09/63	F. Hoffmann-La Roche, Ltd.	2009	2009-2013	virus	Orthopoxvirus	*Vaccinia virus*	modified forms of the E6 and E7 proteins (delE6 and delE7) and the human cytokine IL2 (hIL2)	cancer; infectious disease	cervical intraepithelial neoplasia grade 2 or 3 associated with High Risk HPV infection	
B/BE/09/BVW1	F. Hoffmann-La Roche, Ltd.	2009	2009-2010	virus	Orthopoxvirus	*Vaccinia virus*	modified forms of the E6 and E7 proteins (delE6 and delE7) and the human cytokine IL2 (hIL2)	cancer; infectious disease	cervical intraepithelial neoplasia grade 2 or 3 associated with High Risk HPV infection	
B/NL/08/004	Leiden University Medical Center	2008	2009-2011	bacterium	Lactococcus	*Lactococcus lactis cremoris*	human interleukin-10 (hIL-10) gene	Inflammatory disease	Active Ulcerative Colitis	IIa
B/NL/08/005	Academic Medical Center, Amsterdam	2008	2008-2010	virus	Orthopoxvirus	*Vaccinia virus*	HIV-1 genes env and gag-pol-nef	Infecteous disease	AIDS	II
B/FR/08/09/TG01	MedImmune, LLC	2008	2009-2012	virus	Respirovirus	*Bovine Parainfluenza virus type 3*	Human Parainfluenza virus type 3 (hPIV3) F and HN genes; Respiratory Syncytial virus (RSV) F gene	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3 (PIV3)	I/IIa
B/GB/08/R43/01/S	MedImmune, LLC	2008	2009-2012	virus	Respirovirus	*Bovine Parainfluenza virus type 3*	Human Parainfluenza virus type 3 (hPIV3) F and HN genes; Respiratory Syncytial virus (RSV) F gene	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3 (PIV3)	I/IIa
B/BE/08/BVW1	MedImmune, LLC	2008	2009-2012	virus	Respirovirus	*Bovine Parainfluenza virus type 3*	Human Parainfluenza virus type 3 (hPIV3) F and HN genes; Respiratory Syncytial virus (RSV) F gene	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3 (PIV3)	I/IIa
B/GB/08/R43/01	MedImmune, LLC	2008	2009-2012	virus	Respirovirus	*Bovine Parainfluenza virus type 3*	Human Parainfluenza virus type 3 (hPIV3) F and HN genes; Respiratory Syncytial virus (RSV) F gene	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3 (PIV3)	I/IIa

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/NL/08/006	VU medisch centrum	2008	2008-2011	plasmid DNA	n.a.	n.a.	fibroblast growth factor type 1 (FGF1)	Cardiovascular disease	Critical Limb Ischemia	
B/FI/08/1MA	MedImmune, LLC	2008	2009-2012	virus	Respirovirus	*Bovine Parainfluenza virus type 3*	Human Parainfluenza virus type 3 (hPIV3) F and HN genes; Respiratory Syncytial virus (RSV) F gene	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3 (PIV3)	I/IIa
B/ES/08/49	MedImmune, LLC	2008	2009-2012	virus	Respirovirus	*Bovine Parainfluenza virus type 3*	Human Parainfluenza virus type 3 (hPIV3) F and HN genes; Respiratory Syncytial virus (RSV) F gene	Infecteous disease	Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3 (PIV3)	I/IIa
B/SE/08/EU-2007-006721-27	Ceregene Inc	2008	2008-2010	virus	Dependovirus	*Adeno-associated virus*	human neurturin (NTN) gene	Neurological disease	Parkinson's Disease	II
B/ES/08/47	Universidad de Navarra	2008	2008-2010	virus	Dependovirus	*Adeno-associated virus*	human neurturin (NTN) gene	Neurological disease	Parkinson's Disease	II
B/ES/08/46	Sanofi-Aventis Recherche et Développement	2008	2008-2012	virus	Orthopoxvirus	*Vaccinia virus*	human 5T4 oncofoetal antigen gene	Cancer	Colorectal Cancer	
B/NL/08/003	Stichting Het Nederlands Kanker Instituut	2008	2008-2018	plasmid DNA	n.a.	n.a.	fusion protein domain1 of tetanus toxin fragment C and MART-1(aa 26-35)	Cancer	Melanoma	
B/NL/08/001	Erasmus MC	2008	2008-2011	plasmid DNA	n.a.	n.a.	fibroblast growth factor type 1 (FGF1)	Cardiovascular disease	Critical Limb Ischemia	
B/SE/08/EU-2008-000967-40	ActoGeniX N.V.	2008	2008-2011	bacterium	Lactococcus	*Lactococcus lactis cremoris*	human interleukin-10 (hIL-10) gene	Inflammatory disease	Active Ulcerative Colitis	IIa
B/NL/08/002	Leiden University Medical Center	2008	2008-2018	plasmid DNA	n.a.	n.a.	Human Leukocyte Antigen (HLA)-B7 and β-2 microglobulin	Cancer	Melanoma	III
B/NL/07/011	University Medical Center Groningen	2007	2008-2018	plasmid DNA	n.a.	n.a.	Human Leukocyte Antigen (HLA)-B7 and β-2 microglobulin	Cancer	Melanoma	III
B/NL/07/010	Erasmus MC, University Medical Center Rotterdam	2007	2006-2009	virus	Dependovirus	*Adeno-associated virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Prostate Cancer	III
B/BE/07/BVW3	GENimmune N.V.	2007	2008-2010	virus	Orthopoxvirus	*Vaccinia virus*	synthetic sequence encoding hepatitis B virus (HBV) polyepitope	Infecteous disease	Hepatitis B infection	I
B/BE/07/BVW1	ActoGeniX N.V.	2007	2008-2011	bacterium	Lactococcus	*Lactococcus lactis cremoris*	human interleukin-10 (hIL-10) gene	Inflammatory disease	Active Ulcerative Colitis	Ib/IIa

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/NL/06/010	Raboud University Medical Center	2006	2006-2009	virus	Dependovirus	*Adeno-associated virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Prostate Cancer	III
B/NL/06/011	Academic Medical Center, Amsterdam	2006	2006-2009	virus	Dependovirus	*Adeno-associated virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Prostate Cancer	III
B/NL/06/003	University Hospital Maastricht	2006	2006-2009	virus	Dependovirus	*Adeno-associated virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Prostate Cancer	III
B/NL/06/009	Academic Medical Center, Amsterdam	2006	2006-2008	virus	Orthopoxvirus	*Vaccinia virus*	HIV-1 genes env and gag-pol-nef	Infecteous disease	AIDS	I
B/NL/06/001	University Medical Center Groningen; VU Medical Center	2006	2006-2009	virus	Dependovirus	*Adeno-associated virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Prostate Cancer	III
B/ES/06/42	Oxford BioMedica UK Ltd.	2006	2006-2010	virus	Orthopoxvirus	*Vaccinia virus*	5T4 oncofoetal glycoprotein gene	Cancer	renal adenocarcinoma	
B/SE/06/EU-2006-000985-34	Uppsala University, Division of Clinical Immunology, Uppsala.	2006	2006-2008	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	human CD40L (CD154) gene	Cancer	Urinary Bladder Carcinoma	I/IIa
B/NL/05/006	Erasmus MC, University Medical Center Rotterdam	2005	2006-2008	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	human interleukin-12 gene	Cancer	Prostate Cancer	
B/GB/06/R40/01	Emergent Europe Ltd	2006	2006-2008	bacterium	Salmonella	*Salmonella enterica serovar typhi*	Hepatitis B virus antigen gene	Infecteous disease	Hepatitis B infection	
B/SE/05/151:2005/30190	Swedish institute for Infectious Disease Control	2005	2005-2006	virus	Orthopoxvirus	*Vaccinia virus*	HIV-1 genes env, gag, and pol	Infecteous disease	AIDS	
B/NL/05/001	Academic Medical Center, Amsterdam	2005	2005-2007	virus	Dependovirus	*Adeno-associated virus*	lipoprotein lipase gene	Monogenic disease	LPL deficiency	
B/NL/03/08	VU Medical Center	2003	2004-2006	virus	Dependovirus	*Adeno-associated virus*	human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene	Cancer	Prostate Cancer	I
B/NL/03/02	Erasmus MC, University Medical Center Rotterdam	2003	2004-2006	bacterium	Staphylococcus	*Staphylococcus aureus*	gene encoding a putative cytokeratin receptor (ClfB) insertionally inactivated by introduction of a tetracyclin resistance gene	Infecteous disease		

Notification number	Applicant	Applic .year	Release period	Vector type	Vector genus	Vector species	Insert	Disease type	Disease	Trial phase
B/BE/03/B3	Transgene S.A.	2003	2004-2005	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	human interleukin 2	Cancer	Melanoma	I
B/NL/03/05	International AIDS Vaccine Initiative, New York	2003	2004-2005	virus	Orthopoxvirus	*Vaccinia virus*	HIV-1 gag (structural) protein gene and a series of overlapping epitopes (8-10 amino acids long) from the HIV-1 gag, pol, nef and env proteins.	Infecteous disease	AIDS	I
B/NL/03/01	Academisch Ziekenhuis Leiden	2003	2003-2004	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	*E. coli ntr* gene	other	aseptic prosthetic replacement loosening	I
B/ES/03/35v	Schering Espana SA	2003	2003	virus	Mastadenovirus	*human adenovirus serotype 5 (Ad5)*	fibroblast growth factor 4 gene	Cardiovascular disease	Coronary Artery Disease	
B/GB/03/35/03	Acambis Research Ltd	2003	2003-2006	bacterium	Escherichia	*Escherichia coli*	CS1 antigen gene	Infecteous disease	travellers' diarrhoea	
B/GB/03/R35/02	Acambis Research Ltd	2003	2003-2005	bacterium	Salmonella	*Salmonella enterica serovar typhi*	chromosomal genes aroC, aroD and htrA have been deleted	Infecteous disease	typhoid fever	I
B/GB/02/37/02	Microscience Ltd.	2002	2003-2004	bacterium	Salmonella	*Salmonella enterica serovar typhi*	Hepatitis B virus antigen gene	Infecteous disease	Hepatitis B infection	