National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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Are current EU legislation and risk assessment fit for purpose?

RIVM Letter report 2016-0057
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Colophon

J.W.A. Scheepmaker (author), RIVM
P.A.M. Hogervorst (author), RIVM
D.C.M. Glandorf (author), RIVM

Contact:
Jacqueline Scheepmaker
Department for Gene Technology and Biological Safety
jacqueline.scheepmaker@RIVM.nl

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Synopsis

Future introductions of genetically modified microbial biocontrol agents in the EU
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In the future, genetically modified micro-organisms may offer an alternative to chemical plant protection products. Micro-organisms can be genetically altered to add or enhance certain properties, giving them a wider range of application than regular microbial products. The *Bacillus thuringiensis* bacterium, for instance, can be modified to produce an additional toxin that originates from a related strain. This allows the bacterium to be used as a pesticide against not only harmful caterpillars, but also a harmful species of fly. Organisms may also be modified to retain their effectiveness under unfavourable weather conditions. So far, only a few genetically modified micro-organisms are commercially available outside Europe as plant protection products.

The Dutch government wants to be prepared to deal with companies that seek to obtain EU marketing authorization for such products. Research conducted by the Dutch National Institute for Public Health and the Environment (RIVM) shows that existing EU legislative instruments are sufficient to ensure the safety of such products. The applicable EU legislation provides all the necessary assurances for environmental protection, occupational health and safety, the safety of local residents in agricultural areas, and the main aspects of food and feed safety.

However, there is one (currently hypothetical) situation that is not covered by existing legislation: a genetically modified microbial plant protection product may cause changes in the composition of a food or feed product. This can happen when allergenic or toxic substances are formed due to the effects of a genetically modified micro-organism on plant metabolic processes as a result of the genetic modification. These substances could then end up in food or feed products containing this plant, and could subsequently be harmful after consumption by humans or animals. No examples are currently available. If there are indications that a plant can produce allergenic or toxic substances due to the interaction with the genetically modified micro-organism, it is proposed to take this into account in the risk assessment on a case-by-case basis.

Keywords: genetically modified micro-organisms, microbial plant protection products, safety, EU legislation, environment, food, animal feed, local residents, employees

Publiekssamenvatting

Toekomstige toepassing van genetisch gemodificeerde microbiële gewasbeschermingsmiddelen in de EU
Voldoen huidige EU wetgeving en risicobeoordeling?

Genetisch gemodificeerde micro-organismen zijn in de toekomst mogelijk een alternatief voor chemische gewasbeschermingsmiddelen. Met behulp van genetische modificatie worden eigenschappen van micro-organismen toegevoegd of verbeterd, waardoor ze breder toepasbaar zijn dan 'gewone' microbiële middelen. Zo kan een bacterie *Bacillus thuringiensis* na een aanpassing een extra gifstof produceren van een verwante stam. Dan kan hij niet alleen schadelijke rupsen bestrijden maar ook een schadelijke vlieg. Ook kan het organisme zodanig aangepast worden dat het zijn werkzaamheid onder ongunstigere klimatologische omstandigheden behoudt. Tot nu toe worden maar een paar middelen buiten Europa gebruikt.

Nederland wil erop voorbereid zijn als bedrijven een toelating voor dergelijke middelen tot de Europese markt aanvragen. Uit onderzoek van het RIVM blijkt dat de huidige Europese wettelijke instrumenten toereikend zijn om de veiligheid van dergelijke producten te garanderen. Europese wetgeving dekt de milieuveiligheid, de veiligheid voor omwonenden van landbouwgebieden en voor werknemers volledig af. Ook de belangrijkste aspecten voor voedsel- en veevoederveiligheid worden door Europese wetgeving afgedekt.

Een uitzondering hierop is de hypothetische casus dat de samenstelling van een voedsel- of veevoederproduct wordt veranderd door een genetisch gemodificeerd microbieel gewasbeschermingsmiddel. Dit kan het geval zijn wanneer een genetisch gemodificeerd micro-organisme als gevolg van de modificatie invloed heeft op stofwisselingsprocessen in een plant waardoor allergene of giftige stoffen worden gevormd. Deze stoffen zouden dan in de voedsel- en veevoederproducten kunnen zitten, geconsumeerd kunnen worden en daardoor schadelijk zijn voor mens en dier. Hier zijn echter nog geen concrete voorbeelden van bekend. Voorgesteld wordt om, mochten er aanwijzingen zijn dat een plant gifstoffen of allergenen kan produceren als gevolg van de interactie met het genetisch gemodificeerd micro-organisme, dit van geval tot geval in de risicobeoordeling mee te wegen.

Kernwoorden: Genetisch gemodificeerde micro-organismen, microbiële gewasbeschermingsmiddelen, veiligheid, Europese wetgeving, milieu, voedsel, veevoeder, omwonenden, werknemers

Contents

Summary

Given the rapid developments in new technologies in biocontrol of agricultural crops, applications for the use of genetically (GM) microbial biocontrol agents (MBCAs) may be expected in the EU in the near future. In order to be prepared for future applications in the Netherlands, it is studied in this report whether the current GM legislation and risk assessment sufficiently addresses the potential risks of GM MBCAs for human health, the environment and food and feed derived from plants treated with these GM microbial biocontrol agents (MBCAs).

An inventory of the current legislation applicable to GM MBCAs for agricultural application in the EU shows that most safety aspects of GM MBCAs are covered.
The environmental safety is covered by Regulation (EC) 1107/2009 [1] concerning the placing of plant protection products on the market and Directive 2001/18/EC [2] on the deliberate release into the environment of genetically modified organisms. The safety of workers is covered by Regulation (EC) 1107/2009 and Directive 2000/54/EC [3] on the protection of workers from risks related to exposure to biological agents at work, and the safety of residents, vulnerable groups and bystanders is covered by Regulation (EC) 1107/2009.
However, it was not clear if all aspects concerning the safety of edible food and feed parts derived from crops treated with GM MBCAs were covered by relevant legislation and the respective risk assessment, given the fact that the GM Food and feed Regulation (EC) 1829/2003 [4] is not applicable to GM MBCAs.

In this report therefore two further steps were taken. In the first step, three hypothetical cases were studied. These cases related to plants that were treated with selected GM MBCAs. From this analysis it was concluded that residues of GM MBCAs or their newly expressed GM metabolites may remain on or in the food/feed product and may interact with the food/feed.

In the second step the Food and feed Regulation (EC) 1829/2003 was taken as a starting point. This Regulation covers all relevant data requirements for the safety assessment of GM food/feed. Although this Regulation does not cover food/feed safety of GM MBCAs, it contains all relevant data requirements to assess food/feed safety in an adequate way. Therefore it was considered whether all aspects that are part of the safety assessment of Regulation (EC) 1829/2003 were covered in the risk assessment of Regulation (EC) 1107/2009 and Directive 2001/18/EC.

It is concluded that only in case the GM MBCA or its novel metabolites are capable of changing the composition of the food/feed product there seems to be a potential gap in the risk assessment. This may be the case when the GM MBCA or its newly expressed metabolites interfere with or induce specific pathways, such as those involved in systemic induced resistance or in the formation of antimicrobial metabolites in plants. These pathways may result in the formation of toxic or allergenic

compounds that may impact human and animal safety. It is suggested to include this aspect in the risk assessment of GM MBCAs on a case-by-case basis.

Acknowledgements

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A small international workshop was organized to discuss the findings of this report with experts of the European Food Safety Authority (EFSA), Rijksuniversiteit Groningen (RUG), Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Scientific Institute for Public Health (ISP-WIV) and Perseus. The authors would like to express their gratitude to the experts who participated in the meeting held on 13 June 2016 for their inspiring and fruitful discussions and valuable comments. The input during the workshop was used to adjust and strengthen the report.

Abbreviations

CTGB	Board for the Authorisation of Plant Protection Products and Biocides (Netherlands)
EFSA	European Food Safety Authority
EU	European Union
GM	Genetically modified
GM metabolite	Any metabolite or enzyme that is formed as a consequence of the genetic modification
GMM	Genetically modified microorganism
GMO	Genetically modified organism
ILVO	Institute for Agricultural and Fisheries Research (Belgium)
IPM	Integrated Pest Management
KeMI	Swedish Chemicals Agency
MBCA	Microbial Biocontrol Agent
MRL	Maximum Residue Level
OECD	Organisation for Economic Co-operation and Development
RIKILT	RIKILT Wageningen UR (a Dutch institute for food safety)
RIVM	National Institute for Public Health and the Environment (Netherlands)
RNA	Ribonucleic Acid
RNAi	RNA interference
RUG	University of Groningen (Netherlands)
SM	Secondary metabolite
SUD	Sustainable Use Directive
WIV-ISP	Scientific Institute for Public Health (Belgium)

1 Introduction

In the last decade many biotechnological developments are taking place in agriculture. These developments include genetic modification or gene editing of plants to increase yield, to protect them against biotic and abiotic stresses and the use of RNAi sprays to regulate gene expression in plants. When these biotechnological developments are combined with biological pest control, a rapid development of applications in the field of microbial protection products may be expected in its slipstream. This requires bringing together and integrating expert knowledge on risk assessment of genetically modified organisms (GMOs) and plant protection products.

These developments are also enhanced by the Sustainable Use Directive (SUD) Directive 2009/128/EC [5] that urges member states to intensify integrated pest management (IPM). Some large agrochemical companies already responded to the SUD by acquiring smaller biological companies specialised in MBCAs or started collaborations with companies that manufacture tailor-made micro-organisms capable of controlling pests, diseases or enhancing the uptake of nutrients. With a broader package of pest control products these agrochemical companies meet the IPM policy.

In this report the focus is on the use of microbial biocontrol agents (bacteria, fungi) that are applied onto food and feed crops as living organisms. The drawback in the use of these MBCAs is that their efficacy is not always consistent under field conditions or that they are only effective for limited numbers of crop/pest combinations. MBCAs are therefore specific rather than generic products. Since the nineties of last century research has been performed to improve the efficacy of MBCAs by genetic modification. This can be done by combining traits in one organism or by increasing the persistence of the MBCA or increasing the expression of bioactive components [6]. A few promising products have already been developed [7], but these have never reached the European market. Although the introduction of genetically modified (GM) MBCAs on the European market may still seem far away, applications could be anticipated. The EU project IMPACT [8] already highlighted the progress that has been achieved in the agro-food sector with GM microorganisms. According to this project biotechnology (including genetic modification) can provide new microbial strains which can control disease and stimulate plant growth. This is expected to lead to a reduction in the use of pesticides, fungicides and fertilisers and to provide new options for the control of crop diseases which currently cannot be managed even with existing agricultural chemicals.
The application of a GM MBCA on plants may lead to marketing of food and feed that contain residues of these microorganisms or novel metabolites produced by the GM MBCA as a result of the genetic modification (in this report referred to as GM metabolites), either on the surface of the plant product or in the food/feed itself.

At the moment it is uncertain whether market applications of GM MBCAs will actually be submitted in the EU in the near future. However, the Dutch Ministries concerned with GMOs considered it important to explore in advance, whether the safety of food, feed, human health and the environment treated with these GM MBCAs is adequately covered by existing regulations.

1.1 Goal of this study

This study focusses on the application of living GM MBCAs on food/feed crops in the EU and their potential risks for human health and the environment. Potential effects on operators and workers that come in contact with these products, as well as bystanders and residents, are also included.

The central questions in this report are:

1. What developments are taking place with respect to GM MBCAs?
2. Which legislation is applicable to GM MBCAs for agricultural application in the EU?
3. Do the existing risk assessment methodologies under these EU legislations sufficiently address the potential risks of residues of GM MBCAs and their GM metabolites for food, feed, humans and the environment?

This report firstly maps the developments in GM MBCAs in research and development. Secondly, it describes which legislation is applicable to GM MBCAs for agricultural application in the EU.

Thereafter it was investigated if all necessary aspects in the risk assessment of these GM MBCAs and their application to food/feed crops are actually covered by the applicable risk assessments. Furthermore, potential gaps in the risk assessment are identified.
Two different approaches were chosen to investigate this:

1. On the basis of three case studies, all aspects relevant for the risk assessment of these GM MBCAs were listed, based on expert judgement. Then it was analysed whether all these aspects are indeed covered by the applicable legislations.
2. The second approach was based on the risk assessment performed under Regulation (EC) 1829/2003 that covers GM food and feed safety. If case of data requirements under Regulation (EC) 1829/2003 that were not addressed in either 2001/28/EC or the plant protection regulation it was evaluated whether they are relevant to the safety assessment of GM MBCAs.

2 Overview of the development of genetically modified microbial biocontrol agents

In this chapter an impression is given on the status quo of the development of GM MBCAs worldwide. It needs to be stressed that it is not the intention of this report to give an exhaustive overview. An overall picture is considered to be sufficient to show the ongoing developments. The results of this chapter are also used as a basis for the selection of the three cases that will be dealt with in Chapter 4.

Several sources of information were used (see Appendix 1) to map the developments of GM MBCAs in the world. It was found that only few GM MBCAs were registered on the market outside the EU (Table 4) and that no products were registered in the EU. To give an idea about the developments that take place in this area, an inventory of the stages of development of GM MBCAs (research, patents and field trials in the US and the EU) was made. These are given in Tables 1 to 3.

2.1 Examples of genetic modifications of MBCAs in research and developmental stage

Table 1 gives examples of successful genetic modifications of some well-known MBCAs. This list of examples is not exhaustive but instead gives a snapshot of the available literature. Examples have been retrieved from several key reviews from Gupta and Kindal, 2014 [9], Glare et al. 2012 [7], Klemsdal and Tronsmo, 1999 [10]. Weller and Thomashow, 2015 [11] mention three main categories of genetic approaches of modifications: deletion or mutation of existing genes, alteration of gene Regulation and introduction of heterologous genes (genes from other species).

Table 1. Some reports on successful genetic modifications of well-known MBCAs

Species	**Source**	**Changes in the genome**	**New characteristics**	**Source**
Metarhizium anisopliae	*Metarhizium anisopliae*	Additional copies of the gene encoding cuticle-degrading protease (Pr1)	Hemolymph-induced overexpression of an insect cuticle-degrading protease leading to enhanced virulence	[12]
Pseudomonas fluorescens F113	*Pseudomonas fluorescens*	Mutations in genes *sadB, wspR* and *kinB*	Hypermotility and better root colonisation. As a result, improved biocontrol activity against *Fusarium oxysporum* f. sp. *Radicis-lycopersici* on tomato and	[13]

Species	Source	Changes in the genome	New characteristics	Source
			Phytophthora cactorum on strawberry	
Bacillus subtilis strain ATCC 6633	Constitutive promotor from *Staphylococcus aureus* plasmid pUB110	Replacement of the native promotor of the mycosubtilin operon in ATCC 6633 with a constitutive promoter	Mycosubtilin production leading to improved suppression of *Pythium aphanidermatum* on tomato	[14]
Pseudomonas strain CHAO	Tn5	Repression by bacterial dalicylate and pyoluteorin	Autoinduction of 2,4-Diacetylphloroglucinol biosynthesis leading to enhanced virulence	[15]
Bacillus thuringiensis 3023	*Serratia marcescens*	Introduction of chitinase gene	Stronger biocontrol activity against various pests	[16]
Metarhizium acridium	*Metarhizium robertsii*	introduction of an esterase gene	Enhanced enzyme activity. Expanding the locust specific range to infect caterpillars	[17]
Colletotrichum coccodes	*Fusarium oxysporum*	Introduction of a phytotoxin gene	Reduced moisture requirement, increased virulence and expanded host range	[18]
Metarhizium anisopliae	*Alternaria alternata*	Insertion of dihydroxynaphthalene (DHN) melanin biosynthetic genes	Increased UV tolerance leading to increased survival	[19]
Metarhizium acridium	1. Scorpion *Androctonus australis*, 2. Sydney funnel-web spider *Atrax robustus*, 3. Blue Mountains funnel-web spider *Hadronyche versuta* 4. Australian funnel-web spider *H. versuta*	Insertion of genes expressing four insect specific neurotoxins	Increased virulence leading to higher mortality and reduction of food consumption by locusts	[20]

2.2 Examples of patents

Table 2 gives examples of patents that have been obtained by several large companies.

Table 2. Some relevant patents

Species	What is exposed, effective against	Invention	Origin of genes	Company/Research institute	Patent number and date	Source
Pseudomonas fluorescens strain BL915	phytopathogens	Genes for the synthesis of antipathogenic substances (APS). The invention describes improved biocontrol strains which produce heterologous APSs such pyrrolnitrin and which are efficacious in controlling soil-borne and seedling phytopathogens outside the usual range of the host	*Pseudomonas fluorescens*	Ciba-Geigi Corporation	US 5639949 A	http://www.google.im/patents/US5639949 [21]
Bacillus thuringiensis PS140E2 (B.t. PS140E2), *B. thuringiensis* PS86Q3 (B.t. PS86Q3) and *B. thuringiensis* PS211B2 (B.t. PS211B2)	Ants such as fire ants, carpenter ants, argentine ants, and pharaoh ants	Novel *Bacillus thuringiensis* isolates and toxins with insecticidal activity are described. This invention further concerns genes or gene fragments which have been cloned from novel *B. thuringiensis* isolates which have formicidal activity. These genes or gene fragments can be used to transform suitable hosts for controlling ants	*B. thuringiensis*	Mycogen corporation	US 5616495 A	http://www.google.im/patents/US5616495
B. thuringiensis YBT-881-L1	overexpressed transcription factor CodY protein capable of killing lepidoptera insect of cotton bollworm and citrus fruit flies	Engineered *Bacillus* with a CodY protein		University Huazhong Agricultural	CN102643773 (A) — 2012-08-22 or CN102643773 (B)	[22]

Species	What is exposed, effective against	Invention	Origin of genes	Company/Research institute	Patent number and date	Source
Pseudomonas	Oral nematicide for the control of soil nematodes and plant parasites selected from several nematode genera	Substantially intact, treated cells, having prolonged pesticidal activity when applied to the environment of a target pest, comprising an intracellular polypeptide toxic to the pest, in which the polypeptide is produced as a result of expression of a transformed *Pseudomonas* containing a plasmid comprising a translational enhancer having the sequence TTAATCTAC	*Bacillus*	Mycogen Corporation	EP0471564 A2 or EP0471564A3	http://www.google.im/patents/EP0471564A2?cl=en&hl=nl
Pseudomonas strains, for example strain CGA267356	Plant pathogenic fungi such as *Rhizoctonia* and *Phytium*	Enhanced amounts of secondary metabolites such as pyrrolnitrin, resulting in enhanced biocontrol properties	*P. fluorescens*	Novartis AG, Basle, Switzerland	5,955,348; Sept.21, 1999 Several other related patents: US 5817502 A , EU 0 472 494 and in WO 94/01561	Espacenet

2.3 Overview field trials with GM MBCAs in the USA and in Europe

Table 3 gives an overview of permits for environmental releases in the USA.

Table 3. Permits issued by USDA/APHIS for the environmental release of GM organisms (copied from Hokanson et al. 2014 [23])

	Total # Permits Issued	Years Issued			APHIS Permit No. linked to EA in ISB database
		1985-1994	1995-2004	2005-present	
BACTERIA					
Bt	1				
Bacterium	1				
Clavibacter[a]	7				7[a]
Rhizobium[b]	6				3[b]
Erwinia[c]	3				2[c]
Xanthomonas[d]	15				2[d]
Pseudomonas[e]	26				6[e]
FUNGI					
Cephalosporium stripe	2				
Aspergillus flavus	5				
Fusarium[f]	14				3[f]
Cryphonectria parasitica[g]	5				94-010-01r
Neotyphodium[h]	2				05-152-01r
VIRUS					
TEV (Tobacco etch virus)	1				98-120-01r
Citrus viroid iii	2				99-032-03r
Citrus tristeza virus	5				
Tobacco mosaic virus (TMV)[i]	21				4[i]
INSECTS					
Western orchard predatory mite	1				
Pink bollworm	15				2[j]
NEMATODES					
Heterorhabditis bacteriophora	1				

A note is included if there is more than one description in the database list of organisms, or if there is more than one permit linked to an Environmental Assessment

a *Clavibacter, Clavibacter* xyli 87-355-01r, 88-355-01r, 89-053-01r, 90-016-01r, 90-333-01r, 91-343-01r, 92-329-01r

b *Rhizobium, Rhizobium etli/Rhizobium leguminosarum, Rhizobium etli/Rhizobium leguminosarum/Rhizobium meliloti*, *Rhizobium fredii/Rhizobium leguminosarum* 90-164-03r, 94-207-02r, 97-071-01r

c *Erwinia amylovora, Erwinia carotovora, Pectobacterium carotovorum* 03-279-01r, 05-097-01r

d *Xanthomonas, Xanthomonas campestris, Xanthomonas campestris* pv. *vesicatoria*, Bacterial Spot of Tomato 89-290-01r, 96-071-06r

e *Pseudomonas, Pseudomonas syringae, Pseudomonas syringae pv. syringae, Pseudomonas putida* 90-135-01r, 91-023-06r, 93-026-04r, 95-130-01r, 97-023-02r, 97-023-01r

f *Fusarium graminearum, Fusarium graminearum/Fusarium sporotrichioides, Fusarium moniliforme, Fusarium verticillloides* 94-006-01r, 95-003-01r, 98-355-01r

g *Cryphonectria parasitica*, Chestnut Blight

h *Neotyphodium* sp., *Neotyphodium* sp. Lpl Endophyte

i Tobacco Mosaic Virus (TMV), TMV 91-007-08r, 94-081-01r, 95-041-01r, 96-051-04r

j 01-029-01r, 05-098-01r

In Europe several field trials have been performed. Field trials with GM MBCAs in the EU can only be performed when a permit has been given by the relevant competent authority and these trials are subject to specific conditions. Inspection takes place on a regular basis by the relevant inspection services.
A number of GM microbial inoculants with relevance for food production have been released and tested under commercial field conditions in a number of European countries under the IMPACT project (Interactions between Microbial inoculants and resident Populations in the rhizosphere of Agronomically important Crops in Typical soils), an EU-funded research project [8]. Three examples are given below.

Genetically modified strains of *Pseudomonas fluorescens* F113 were developed with overproduction of the antifungal metabolite phloroglucinol (Phl). A trial was conducted to determine, among others, whether the GM strain had a negative effect on the environment (e.g. native indigenous microorganisms, arbuscular mycorrhizal fungi, persistence in soil) and whether the strain effectively controlled damping-off disease compared to the chemical fungicide.
Genetically modified *Azospirillum brasilense* Sp6 strain producing elevated levels of the plant growth stimulating factor IAA (Indole-3-acetic acid, a plant growth promoting hormone) was tested in the field, also under the IMPACT project. The growth in soil, effect on the grain yield of sorghum and effects on the indigenous microbial population were assessed.
Field trials with genetically modified *Pseudomonas putida* WCS358r have been performed in the Netherlands. This strain was modified to produce the antifungal metabolites phenazine and phloroglucinol to suppress fungal pathogens on wheat. The trials were conducted to assess potential negative impacts on the rhizosphere microflora of wheat [24, 25].

2.4 Examples of registrered GM MBCAs

In Table 4 an overview is given of registered GM MBCAs. For this purpose databases of the regulatory agencies in the USA, Canada, Australia and the EU, have been searched (see Appendix 7 for sources of information). Registered products were only found in the USA of which Nogall is also registered in Australia. One product is based on a strain of *Agrobacterium radiobacter* (NOGALL), two products are based on strains of *Bacillus thuringiensis* (Crymax WDG/WP, Lepinox WEG/G bioinsecticide) and one product is based on a strain of *Pseudomonas fluorescens* (Frostban B).
In the USA more products have been registered before (see Appendix 8). According to C. Wozniak (EPA, pers. comm.), their withdrawal was caused by discontinuation of the payment of the registration fees. The exact reasons for withdrawal are unknown to the EPA.

Table 4. Overview of current approvals

Trade name and ID-number	**Notification number**	**Recipient organism**	**New characteristic**	**Company**	**Reference**
NOGALL	EPA Reg. No. 62388-1 Australia: permit nr. PER13150	*Agrobacterium radiobacter* strain K1026	Deletion of a fragment producing an immunity in the pathogen[1]	BASF AGRICULTURAL SPECIALTIES PTY LTD	[10]; http://www.newbioproducts.net/nogall-.html; [24], [2], 21][2]
CRYMAX™ WDG/WP bioinsecticide	CryMax, EPA Reg. No. 70051-86; CryMax WP, EPA Reg. No. 70051-90	*Bacillus thuringiensis* strain EG7841	Cry 1c protein from *B. thuringiensis* var. *aizawai*	Certis USA, LLC	[28], [29][2]
Lepinox™ and Lepinox™ WDG bioinsecticide	Lepinox, EPA Reg. No. 70051-87; Lepinox WDG, EPA Reg. No. 70051-89	*Bacillus thuringiensis* strain EG7826	Cry 1Ac/1F[3] protein from *B. thuringiensis* var. *kurstaki / aizawai*	Ecogen/ Certis	[28], [29][2]
Frostban B = BlightBan A506	EPA Reg. No. 228-710	*Pseudomonas fluorescens* A506	Protein for ice-nucleation has been deleted: reduction of frost damage	NuFarm Americas, Inc.	[29][2]

1: A toxic compound produced by both K1026 and K84 controls certain other A*grobacterium* spp. that causes crown gall disease.
2: derived from Table 4.1 from [29] and a check on the current regulatory status d.d. 24-2-2015 (personal comm. Wozniak)

3 Relevant EU Regulations and Directives and their scopes

In this chapter an overview is given of the EU legislation that may be relevant for placing a GM MBCA on the European market for agricultural applications, with respect to their safety for human health, the environment and food/feed treated with these GM MBCAs. Also legislation covering the safety of operators and workers that come in contact with the GM MBCAs, as well as bystanders and residents, is included.

3.1 Directive 2001/18/EC for the deliberate release of genetically modified organisms into the environment

The protection of human health and the environment requires that due attention be given to controlling risks from the deliberate release into the environment of genetically modified organisms (GMOs). The EU has consequently adopted a legislative framework on the deliberate release of GMOs into the environment and the placing of GMOs on the market in accordance with the precautionary principle. This framework provides authorization procedures, a common methodology for risk assessment and a safety mechanism.

A GM MBCA falls under the definition of a GMO and therefore consent under (part C of) this Directive is necessary before a GM MBCA can be placed on the EU market.

This Directive covers:

- a procedure for granting consent for the deliberate release and placing on the market of GMOs;
- a common methodology to assess case-by-case the risks for the environment associated with the release of GMOs;
- a monitoring obligation after their deliberate release;
- a mechanism allowing the release of the GMOs to be modified, suspended or terminated where new information becomes available on the risks of such release;
- inspections and other control measures as appropriate;
- measures to ensure traceability of GMOs.

Before submitting a notification und part C (placing a GM MBCA on the market) of Directive 2001/18/EC an environmental risk assessment needs to be carried out in accordance with the principles set out in Annex II to this Directive and on the basis of the type of information specified in Annex III to this Directive.

With respect to animal health, According to Annex II, D.1.7 the following will be analysed in the environmental risk assessment:
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.

In practice, this analysis is limited to (direct) toxic or allergenic effects resulting from incidental or accidental consumption of the GM MBCA (not chronic consumption).

With respect to human health, According to Annex II, D.1.7 the following will be analysed in the environmental risk assessment:
Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GM MBCA and persons working with, coming into contact with or in the vicinity of the GM MBCA release(s).
In practice this analysis is limited to (direct) toxic or allergenic effects resulting from incidental consumption of the GM MBCA, or by handling the GM MBCA.

In Annex III A (GMOs other than higher plants) the following information is required under "considerations for human health and animal health, as well as plant health" (C.2.i.):

(i) toxic or allergenic effects of the GMOs and/or their metabolic products;
(ii) comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
(iii) capacity for colonization;
(iv) if the organism is pathogenic to humans who are immunocompetent:
- diseases caused and mechanism of pathogenicity including invasiveness and virulence;
- communicability;
- infective dose;
- host range, possibility of alteration;
- possibility of survival outside of human host;
- presence of vectors or means of dissemination;
- biological stability;
- antibiotic resistance patterns;
- allergenicity;
- availability of appropriate therapies.

(v) other product hazards.

3.2 Regulation (EC) 1107/2009 for plant protection products

This Regulation lays down the rules for the authorization of plant protection products in commercial form and for their placing on the market, use and control within the EU. This Regulation increases the level of health and environmental protection, contributes to better protection of agricultural production, enlarges and consolidates the internal market for plant protection products.

The scope of this Regulation covers plant protection products, their active substances and their residues.

This Regulation covers:

- All aspects of the risk assessment of the GM MBCA in the environment;
- Residues in food and feed;

- Risk assessment of professional or non-professional users, bystanders, workers, residents, specific vulnerable groups or consumers, directly or indirectly exposed through food, feed, drinking water or the environment.

A microorganism is defined as any microbiological entity, including lower fungi and viruses, cellular or non-cellular, capable of replication or of transferring genetic material.

Residues are defined as 'one or more substances present in or on plants or plant products, edible animal products, drinking water or elsewhere in the environment and resulting from the use of a plant protection product, including their secondary metabolites, breakdown or reaction products'. This definition is used for both chemical as microbial biological pesticides.

Therefore, under Regulation (EC) 1107/2009 all metabolites of the GM MBCA (normal metabolites and the GM metabolites) have to be assessed. If a GM MBCA is used as or in a plant protection product, it needs to comply with Regulation (EC) 1107/2009.

A plant protection product which contains an organism falling within the scope of Directive 2001/18/EC shall be examined with respect of the genetic modification in accordance with that Directive, in addition to the assessment under Regulation (EC) 1107/2009. Section 48 in Regulation (EC) 1107/2009 states that "authorization under this Regulation shall not be granted for such a plant protection product unless written consent, as referred to in section 19 of Directive 2001/18/EC, has been granted for it". Thus, for placement of a GM MBCA on the market it needs to comply with both Directive 2001/18/EC and with Regulation (EC) 1107/2009. An evaluation and consent under Directive 2001/18/EC must be obtained first, before proceeding to an evaluation and consent under Regulation (EC) 1107/2009.

3.2.1 Commission Regulation (EU) 283/2013 and 284/2013

The data requirements under Regulation (EC) 1107/2009 for the active substance and the product are set out in Commission Regulation (EU) 283/2013 [30] and Commission Regulation (EU) 284/2013 [31], respectively.

IIB on micro-organisms including viruses (Commission Regulation (EU) 283/2013)

1. Identity
2. Biological properties
3. Further information on the micro-organism
4. Analytical method
5. Effects human health
6. Residues in or on treated products and feed
7. Fate and behavior in the environment
8. Effects on non-target organisms
9. Summary and evaluation of environmental impact

IIIB on the product (Commission Regulation (EU) 284/2013)

1. Identity of the plant protection product
2. Physical, chemical and technical properties of the plant protection product
3. Data on application
4. Further information on the plant protection product
5. Analytical methods
6. Efficacy data
7. Effects on human health
8. Residues in or on treated products and feed
9. Fate and behavior in the environment
10. Effects on non-target organisms
11. Summary and evaluation of environmental impact

3.3 Regulation (EC) 1829/2003 on genetically modified food and feed

As indicated before, this Regulation is not applicable for GM MBCAs. However, this regulation is included here because we refer to this Regulation in relation to the use of GM MBCAs in Chapter 5.

The objective of this Regulation is to:

1. provide the basis for ensuring a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to genetically modified food and feed, whilst ensuring the effective functioning of the internal market
2. lay down Community procedures for the authorization and supervision of genetically modified food and feed;
3. lay down provisions for the labelling of genetically modified food and feed.

Article 16 of Regulation (EC) 1829/2003 says:
This Regulation should cover food and feed produced 'from' a GMO but not food and feed 'with' a GMO. The determining criterion is whether or not material derived from the genetically modified source material is present in the food or in the feed. Processing aids which are only used during the food or feed production process are not covered by the definition of food or feed and, therefore, are not included in the scope of this Regulation. Also food and feed which are manufactured with the help of a genetically modified processing aid are not included in the scope of this Regulation.

It can be concluded from Article 16 that a GM MBCA is not considered to be a food/feed item by itself and will not be assessed under this Regulation.

3.3.1 EFSA Scientific opinion – Guidance on the risk assessment of GM microorganisms and their product intended for food and feed use

On page 5 of this scientific opinion of the European Food Safety Authority (EFSA) it is written: "GMMs used as plant protection products or biocides, fall within the scope of the Directive 2001/18/EC and such microorganisms are not considered food or feed and, therefore, are not covered by this guidance document."

This confirms the conclusion drawn from article 16 of Regulation (EC) 1829/2003 that GM MBCAs used as or in a plant protection product are not considered a food or feed and are not covered by Regulation (EC) 1829/2003.

3.4 Regulation (EC) 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin

For food and feed products produced in the EU, Regulation (EC) No 396/2005 is used in combination with Regulation (EC) 1107/2009.

Definitions used in Regulation (EC) No 396/2005

'**Maximum residue level' (MRL)** means the upper legal level of a concentration for a pesticide residue in or on food or feed that is set, based on good agricultural practice and the lowest consumer exposure necessary to protect vulnerable consumers.

'**Pesticide residues'** are defined as residues, including active substances, SMs and/or breakdown or reaction products of active substances currently or formerly used in plant protection products as defined in article 3, point 1 of Regulation (EC) 1107/2009, which are present in or on the products covered by Annex I to Regulation (EC) 396/2005, including in particular those which may arise as a result of use in plant protection, in veterinary medicine and as a biocide.

Regulation (EC) 1107/2009 states in Article 29 'for plants or plant products to be used as feed or food, where appropriate, the maximum residue levels (MRL) for the agricultural products affected by the use referred to in the authorisation have been set or modified in accordance with Regulation (EC) No 396/2005'. This implicates that Regulation (EC) No 396/2005 covers the data requirement of residues of GM MBCAs in food and feed and is thus relevant for food and feed that has been treated with GM MBCAs as it sets maximum levels of pesticides in products of plant and animal origin.
For import of food and feed, Regulation (EC) No 396/2005 is used independently of Regulation (EC) 1107/2009.

Regulation (EC) No 396/2005 contains a list of active substances that do not require an MRL (*Ampelomyces quisqualis* strain AQ10, *Bacillus subtilis strain* QST 713, *Coniothyrium minitans* strain CON/M/91-08 (DSM 9660), *Gliocladium catenulatum* strain J1446 *Paecilomyces fumosoroseus* apopka strain 97 and *Pseudomonas chlororaphis* strain MA342). Any GM MBCA that would be developed based on one of these strains will obtain a new strain number and would thus fall under Regulation (EC) No 396/2005. The MRL (default 0.01 mg/kg) is however only suitable for chemical active substances and no suitable for microorganisms and their metabolites as these cannot be expressed in mg/kg (usually in Colony Forming Units/g soil).

3.5 Commission Regulation (EC) 1881/2006 setting maximum levels for certain contaminants in foodstuffs

This Regulation applies to microorganisms that are human pathogens. GM MBCAs are generally not human pathogens, and when they are, they

will not be approved under the Plant Protection Products Regulation (EC) 1107/2009. For completeness, information on this Regulation is included in this chapter.

In order to protect public health, contaminants should be kept at levels at which they are toxicologically acceptable. For this reason Commission Regulation (EU) 1881/2006 [32] sets maximum levels (MLs) for contaminants in foodstuffs. MLs are amended regularly for certain contaminants to take into account new information and developments in the Codex Alimentarius[1].

3.6 Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work

This Directive protects the health and safety of workers exposed to biological agents whilst undertaking their work and lays down rules concerning risk assessment and limitation if such exposure cannot be avoided.

Biological agents are defined as 'micro-organisms', including those which have been genetically modified, cell cultures and human endoparasites, which may be able to provoke any infection, allergy or toxicity.

Dir. 2000/54 EC provides a list known pathogens in humans

In this list, pathogens are classified into four risk groups, according to their level of risk of infection:

1. group 1 biological agent means one that is unlikely to cause human disease;
2. group 2 biological agent means one that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available;
3. group 3 biological agent means one that can cause severe human disease and present a serious hazard to workers; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available;
4. group 4 biological agent means one that causes severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community; there is usually no effective prophylaxis or treatment available.

Pathogenicity may be caused by pathogenic or virulence factors of the organism itself. Toxicity can be caused by the SMs produced by the micro-organism. Metabolites are often produced during fermentation in the growing medium. The array of SMs being produced depends on factors such as temperature, pH and composition of the growing medium. It is possible to steer the production process and it is also

[1] Codex standards are recommendations for voluntary application by members, but in many cases they serve as a basis for national legislation. Codex committees, when developing standards, apply risk analysis and rely on the independent scientific advice provided by expert bodies organized by FAO/WHO. These bodies also give direct advice to Member Governments.

possible to exclude SMs from the end product for example by sieving the spores. Nevertheless, SMs can be included in the end product and workers can be exposed to them during the process of formulation or application.

Thus, a GM MBCA does need to comply with Directive 2000/54/EC as a GM MBCA is potentially pathogenic to workers and the SMs are potentially toxic to the workers. It is noted that all aspects covered by Directive 2000/54/EC also fall under Regulation (EC) 1107/2009.

3.7 First conclusions on the scopes of the relevant legislation

In Table 5 an overview is given of the scopes of the Directives and Regulations that were found relevant regarding GM MBCAs in the preceding paragraphs. The scopes can be divided into five distinct groups (aspects of risk assessment):

- The environment;
- Residues in food/feed of the GM MBCA and its metabolites (including those that are made in addition due to the genetic modification);
- Safety of food/feed itself that is treated with GM MBCAs due to a potential change in composition because of the treatment. This aspect was identified in 3.3;
- Bystanders, residents, specific vulnerable groups;
- Operators and workers. An operator is defined as the person who is involved with formulation procedures and performance of the application. A worker is defined as the person who is handling the treated crops and products.

Table 5. Overview of the scopes of applicable regulations

	Scope	**Aspects of risk assessment**				
Legislation		**Environment**	**Residues GM MBCAS on food/feed**	**Food/feed (composition of food/feed)**	**Bystanders, residents, specific vulnerable groups**	**Operators, workers**
Directive 2001/18/EC	Release of genetically modified organisms (GMOs) in the environment	√	√	X	X	X
Regulation (EC) 1107/2009	Plant protection products	√	√	X	√	√
Directive 2000/54/EC	Protection of workers	X	X	X	X	√
Regulation (EC) 396/2005		X	√	X	X	X

√ : covered under scope
X : not covered under scope

Table 5 shows that:

- Environmental safety aspects of the application of GM MBCAs are covered, in both Directive 2001/18/EC and Regulation (EC) 1107/2009;
- Food/feed safety of residues of GM MBCAs and its metabolites on/in food/feed are covered, in both Directive 2001/18/EC and Regulation (EC) 1107/2009;
- Safety assessment of the food or feed with respect to the composition of the food/feed after treatment with the GM is not addressed in Directive 2001/18/EC and Regulation (EC) 1107/2009;
- Bystanders and other groups are covered by Regulation (EC) 1107/2009;
- Operators and workers are covered by Regulation (EC) 1107/2009 and Directive 2000/54/EC.

Based on this analysis it seems that there is only a gap in legislation regarding the safety of food/feed treated with GM MBCAs. Food and feed safety of GMOs is generally covered by Regulation (EC) 1829/2003, but this Regulation is not applicable to GM MBCAs. Directive 2001/18/EC and Regulation (EC) 1107/2009 cover certain aspects of the food and feed safety assessment, but this seems only to be the case for the safety of GM MBCA itself (and its residues) and not for the food/feed, which may be affected by the GM MBCA or its metabolites by induction of certain metabolic pathways.

From this overview it cannot be concluded whether the food and feed safety is sufficiently covered. Therefore, this aspect is investigated in more detail in the next two chapters.
However, in Chapter 9 (Appendix 3) it is first investigated whether residues of GM MBCAs could actually be present in or on food/feed derived from crops that were treated with these GM MBCAs and could affect food/feed safety in order to take this following step.

4 Food and feed safety assessment of food/feed crops treated with GM MBCAs on the basis of three cases

In the previous chapter a potential gap in the legislation regarding the safety of food/feed treated with GM MBCAs was identified with respect to a potential change in composition of the food/feed itself as a consequence of the GM MBCA treatment. Although not covered by Regulation, it could be the case that this aspect is already taken into account in the actual risk assessment of the GM MBCA under either Regulation (EC) 1107/2003 or Directive 2001/18/EC. In this chapter this is studied on the basis of three hypothetical cases.

4.1 Description of exercise

Three (hypothetical) cases were selected based on microorganisms that are widely used in biocontrol (see Chapter 2). Each case pictures the interaction between the GM MBCA in question, the way it is applied, and the pathogen that is intended to be suppressed or killed. In this exercise these three cases are used to work through all the relevant questions for a risk assessment with regard to the safety of the food/feed product that has been treated with the selected GM MBCA. The goal of this exercise was twofold:

1. We considered whether residues of GM MBCAs and their metabolites could actually be present on the food/feed. Only in that case a potential interaction with the food/feed can be expected. To answer this question, expert judgement was used;
2. It was analysed if the potential gap in legislation for food/feed safety identified in the previous chapter was also identified when performing a risk assessment.

The selection of the cases is based on two criteria. Firstly, they are representatives of the major groups of biocontrol agents and therefore expected to be likely candidates for genetic modification in the future. This is confirmed by Weller and Tomashow, 2015 [11], who state that microorganisms that are most promising for future development as transgenic MBCAs are *Trichoderma, Beauveria, Metarhizium* and *Bacillus.* Secondly, the way these GM MBCAs are applied to crops is different (*e.g.* spray, soil drench, seed coating). This will result in differences in colonization and survival of the biocontrol agents on the crop plants and in the environment. It was attempted to select real cases that are already tested in field trials. This was not possible and some cases are therefore (still) hypothetical. As the purpose of this report is to identify potential gaps in relevant regulations and risk assessment procedures of GM MBCAs, this was not considered to be a problem.

Pseudomonas putida, *Beauveria bassiana* and *Bacillus thuringiensis,* respectively, were selected MBCAs. Relevant genetic modifications and the relevant crops are described in Appendix 3.

The three cases were each submitted to a list of questions (see Table 8,

Table 9 and Table 10 in Appendix 3). As safety of food/feed treated with GM MBCAs was identified to be a possible gap in the legislation applicable for GM MBCAs, the questions on the three cases will have a strong focus on this issue. The purpose was to predict whether residues of GM MBCAs can remain on or in food/feed products treated with these GM MBCAs and to verify if all the relevant questions in this risk assessment exercise are covered by the existing legislation.

Questions in the table are the same for each case and are grouped under three different headings:
Biocontrol product, ***the organism*** and ***the metabolites formed by the GM organism*** in order to address all possible aspects of the fate and survival of the biocontrol product/micro-organism/SMs.

The answers are based on a worst case scenario unless it directly follows from the nature of the organism or the genetic modification that the probability of this event to take place is negligible. A worst case scenario assumes a 100% probability of the event to occur.

4.2 Results

Based on this exercise, two main conclusions can be drawn with regard to the food and feed safety:

1. a GM MBCA can persist as a residue on or in the food/feed product and can potentially affect the food/feed safety of the product;
2. the metabolite(s) of the GM MBCA produced as a consequence of the genetic modification can persist in or on the food/feed product and can potentially affect the food/feed safety of the product.

Appendix 3 also shows that the most important aspects of food and feed safety with respect to the GM MBCA and its metabolites are addressed in the applicable legislation.

5 Examination of risk assessment requirements of Regulation (EC) 1829/2003 on genetically modified food and feed

5.1 Description of the exercise

It was concluded in Chapter 3 that Regulation (EC) 1829/2003 on genetically modified food and feed is not applicable to the evaluation of food/feed obtained from plants treated with GM MBCAs. Directive 2001/18/EC and Regulation (EC) 1107/2009 were found to cover certain aspects of the food and feed safety assessment, but it was not certain whether this was sufficiently covering the food/feed safety. From Chapter 4 it was evident that GM MBCAs applied to food and feed crops, as for any MBCA, may indeed remain as residues in or on the harvested food/feed.

We took a further step in order to look at aspects that are taken into account in the actual risk assessment of food and feed under the relevant legislation of GM MBCAs compared to that in the GM food feed safety. For this, the data requirements of (EC) 1829/2003 on genetically modified food and feed were compared with the data requirements of Regulation (EC) 1107/2009 on plant protection products and Directive 2001/18/EC on the deliberate introduction in the environment of GMOs with respect to food/feed treated with GM MBCAs, and potential differences were identified.

In Appendix 4 the data requirements of Regulation (EC) 1829/2003[2] are listed in the first column of Table 11. In the next two columns of this table it is indicated if these aspects are covered by the risk assessment under Directive 2001/18/EC or Regulation (EC) 1107/2009, respectively. By assuming that Regulation (EC) 1829/2003 covers all relevant aspects of the food and feed safety assessment of GM food and feed, any difference indicated in this table could reveal a potential gap with respect to the safety assessment of food and feed treated with GM MBCAs.

A second step in this exercise was to analyse if the potential gaps in the applicable legislation, based on the data requirements of Regulation (EC) 1829/2003 are also relevant to food/feed treated with GM MBCAs. This was done in the second part of Appendix 4.

5.2 Results

Table 11 in Appendix 4 showed the following results:

Comparative analysis

Under Regulation (EC) 1829/2003 for GM food/feed a compositional analysis is performed of the GM food/feed in comparison to the non-GM food/feed to screen for potential (un)intended effects of the genetic modification. Under Directive 2001/18/EC and Regulation (EC) 1107/2009 only the residues of the GM MBCA itself and their

[2] The data requirements are set in Commission implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorization of genetically modified food and feed in accordance with Regulation (EC) 1829/2003 of the European Parliament and of the Council.

metabolites are analysed and not the (edible)food/feed parts that have been treated with the GM MBCAs, such as tomatoes or sweet corn.

It is concluded that there is a gap in the risk assessment with respect to the compositional analysis of food/feed parts that have been treated with GM MBCAs.

Toxicology
Under the Food and feed Regulation (EC) 1829/2003 it is, among others, assessed whether the introduced sequences may be toxic, by using bioinformatics. Only for new proteins, besides a 28 day study, also an animal study is requested. With respect to the bioinformatic analyses, the sequences of the newly inserted DNA of the junction regions between insert and genomic DNA and of the newly expressed proteins are determined and compared to sequences of known toxins. It is also determined if endogenous genes are disrupted. In case no similarity to known toxins is found and no known endogenous genes are disrupted, no further data are required. If there are indications for toxicity, either from the 28-day studies or the bioinformatic analyses, further tests have to be supplied. Under Directive 2001/18/EC this is assessed in the same way, but only for the GM MBCA itself and not for the food/feed treated with the GM MBCAs. Also under Regulation (EC) 1107/2009 toxicity of the GM MBCA is assessed. The potential toxicity of the whole food/feed that may contain residues of GM MBCAs is not assessed under the two latter regulations.

It is concluded that there is no gap in the risk assessment with relation to toxicology of the GM MBCA itself. However, a gap in the risk assessment may exist with respect to potential toxicity of the food/feed treated with the GM MBCAs.

Allergenicity
Under the Food and feed Regulation (EC) 1829/2003 it is assessed, among others, whether the introduced sequences may lead to allergenicity of the food/feed product by using bioinformatics. The sequences of the newly inserted DNA, its bordering regions (junction between insert and genomic DNA) and of the newly expressed proteins are determined and compared to sequences of known allergens. In case there is no similarity to known allergens, no further data are required. If there are indications for allergenicity, further tests have to be supplied. Under Directive 2001/18/EC the GM MBCA is evaluated in the same way, but the food/feed treated with the GM MBCAs is not evaluated under Directive 2001/18/EC.
Under Regulation (EC) 1107/2009 allergenicity of the GM MBCA is evaluated using relevant clinical observations or animal studies. These tests are only focused on dermal and inhalation allergies, not on food allergens. Bioinformatic studies are not performed under Regulation (EC) 1107/2009. No further studies are requested with respect to the food feed treated with the GM MBCAs.

It is concluded that there seems to be no gap in the risk assessment with relation to the allergenicity of the GM MBCA itself. However, a gap

in the risk assessment may exist with respect to potential allergenicity of the food/feed treated with the GM MBCAs toxicity.

Overall conclusion

This exercise in which data requirements under Regulation (EC) 1829/2003 were compared with those of the Regulations applicable to GM MBCAs, demonstrated that potential risks of toxins or allergens produced by residues of GM MBCAs in or on food/feed are adequately covered in the assessment of GM MBCAs under Directive 2001/18/EC and Regulation (EC) 1107/2009. However, there is a difference with respect to the assessment of the food/feed product itself. Under Regulation (EC) 1829/2003 the GM food/feed product itself is assessed with respect to potential toxicity and allergenicity that could arise as a consequence of the genetic modification. This is not the case under Directive 2001/18/EC and Regulation (EC) 1107/2009 where only the safety of the residues of the GM MBCAs is assessed and not the safety of the food/feed product it was applied to. Microbial inoculants are known to interact closely with plants and to induce for example disease resistance. It is possible that the GM MBCA or its novel metabolites induces (or interact with) specific metabolic pathways in the plants, potentially leading to the formation of metabolites in the food that are toxic or allergenic to humans and animals. It is suggested to take this aspect into account in the safety assessment of GM MBCAs on a case-by-case basis.

6 Discussion and conclusions

Discussion
The overview in Chapter 2 on GM MBCAs shows that commercial application of GM MBCAs is currently at a very low level and so far there have been no commercial applications in the EU. However, an increase in the agricultural application of these products is to be expected driven by the Sustainable Use Directive that urges member states to intensify integrated pest management. In that respect also GM MBCAs biocontrol agents may be expected to reach the market for their agricultural application in the future.

We answered the question whether current EU legislation sufficiently covers all safety aspects of GM MBCAs. It was concluded that environmental safety, safety of workers, residents, vulnerable groups and bystanders are sufficiently covered. However, a gap in the legislation regarding the safety of food/feed treated with GM MBCAs was identified, as Regulation (EC) 1829/2003 on food and feed safety of GMOs is not applicable to GM MBCAs and on food/feed treated with these GM MBCAs.

Further investigation at the risk assessment level indicated that the safety of residues of GM MBCAs and their newly produced metabolites (as a consequence of the genetic modification) on food and feed are adequately covered by the risk assessment under Directive 2001/18/EC and Regulation (EC) 1107/2009, but this is not the case for the safety of the food or feed product itself. This was flagged as a gap. It should be mentioned that this same gap is also applicable to non-GM MBCAs.

Micro-organisms are known to interact with plants and are able to induce specific pathways in plants, such as those involved in induced systemic resistance of plants [33] or those involved in the formation of of secondary metabolites in plants such as fytoalexins [34]. The question is if the identified gap is relevant from a viewpoint of risk assessment.

It can be argued that the need for safety assessment of food or feed from plants treated with GM MBCAs can be scientifically justified in case these GM MBCAs or their novel GM metabolites are capable of changing the composition of the food/feed product. This may be the case when the GM MBCA (or its novel GM metabolites) interfere with or induce specific metabolic pathways in the plant (as described above), resulting in the formation of toxic or allergenic compounds. However, the exercise with the three cases indicated that in general only low amounts of the GM MBCA or its novel GM metabolites will be present in or on the food/feed product. It is not very likely that this will lead to a change in the composition of the food or feed product. On the other hand this is not excluded and the interaction between the GM MBCA and the plant, leading to a changed composition of the plant, could have occurred at an earlier stage such as during application. This could be assessed on a case-by-case basis, depending on the GM MBCA and its genetic modification.

Interaction of the MBCA with the food/feed product could equally be true for non-GM MBCAs exhibiting an increased production of metabolites induced by classical mutagenesis. However, this is currently not taken into account under Regulation 1107/2009.

The difference between Regulation 1107/2009 and Directive 2001/18/EC is that under the Regulation the environmental risk assessment is applicable to the MBCA, including all its metabolites. In the environmental risk assessment under Directive 2001/18/EC effects of the GM MBCA is compared with those of its non-GM counterpart. This means that potential effects of the GM micro-organism are set against a baseline. This baseline is in case of a GM MBCA, the impact of the (non-GM) MBCA and its metabolites.

We do not imply that the interaction of residues of (GM) MBCAs or their GM metabolites with food and feed products would lead to a safety issue of the food or feed and that regulation has to be adjusted in that respect. Our suggestion would be to take this aspect into account only in case there is a scientific trigger to do so. If such a trigger becomes apparent in the risk assessment of a GM MBCA under Directive 2001/18/EC, this could be flagged up so that this aspect can also be taken into account in the subsequent assessment under Regulation (EC) 1107/2009 which normally does not address this aspect.

In this report we have not addressed import of food/feed that is treated with GM MBCAs. It is expected that GM MBCAs are already applied outside the EU or will be in the near future. In that case Regulation (EC) 1107/2009 is not applicable. If the food/feed is known to contain living GM MBCAs this has to be assessed under Directive 2001/18/EC. However, treatment of GM MBCAs may not always be reported. For these products only the residue legislation Regulation (EC) 396/2005 is applicable, which in case of import can be used independently of Regulation (EC) 1107/2009. For micro-organisms this residue legislation is not useful as its criterion, the MRL (0.01 mg/kg), is not applicable to microorganisms. This regulation does therefore not adequately assess the safety aspects related to imported food or feed that has been treated with GM MBCAs.

Conclusions

It can be concluded that in case food/feed crops are treated with GM MBCAs in the EU, the safety of GM MBCAs for human health and the environment is covered by relevant legislation and the current applicable risk assessment strategies. Also the food/feed safety of residues of the GM MBCAs and their new metabolites remaining on food/feed are covered. We observed one gap. Unlike the assessment of GM crops under Regulation (EC) 1829/2003, which involves a broad compositional analysis (fatty acids, vitamines, proteins, etc.), the food/feed products treated with GM MBCAs are not assessed under the relevant legislation (Directive 2001/18/EC and Regulation (EC) 1107/2009) with respect to their food and feed safety. This is only relevant in case an interaction is expected between the GM MBCA residue or its novel metabolite and the crop plant, for example by inducing or interfering with metabolic pathways of the plant which could lead to toxin or allergen formation. We advocate that only in case there is a scientific trigger to assume that

the GM MBCA or its novel metabolites could affect the composition of the food or feed resulting in potential toxicity or allergenicity, this aspect is to be taken into account in the safety assessment.

7 Appendix 1: Sources of information

7.1 General information sources on the status of GM micro-organisms in the EU

Joint Research Centre (JRC), Deliberate Release and Placing on the EU market of GMOs – GMO register
The purpose of this web site, managed by the Joint Research Centre of the European Commission on behalf of the Directorate General for Health and Consumers is to publish information and to receive comments from the public regarding notifications on GMO's about deliberate field trials and placing on the market of genetically modified organisms, as defined in Directive 2001/18/EC.

7.2 General information sources on the status of GM micro-organisms worldwide

The Biosafety Clearing-House [35]
BCH is a site set up by the Cartagena Protocol on Biosafety to facilitate the exchange of information on Living Modified Organisms (LMOs) and assist the Parties to better comply with their obligations under the Protocol. Global access to a variety of scientific, technical, environmental, legal and capacity building information is provided in the six official languages of the UN.

Environmental protection Authority [36]

The EPA of New Zealand provides information on new organisms (including GM organisms).

Information systems for biotechnology, A National Resource in Agrobiotech Information [37]
ISB provides information resources to support the environmentally responsible use of agricultural biotechnology products. Here, documents can be found and searchable databases pertaining to the development, testing and regulatory review of genetically engineered (GE) plants, animals and microorganisms within the United States and Hawaii.

Office of the Gene Technology Regulator [38]
The OGTR provides a list of applications and licenses for Dealings involving Intentional Release of GMOs into the environment in Australia. The OGTR has been established within the Australian Government Department of Health and Ageing to provide administrative support to the Gene Technology Regulator in the performance of his functions under the *Gene Technology Act 2000*. This office provides GMO records on all approved GMOs and GM products in Australia.

Australian Pesticides and Veterinary Medicines Authority [27]

U.S. Environmental Protection Agency (U.S. EPA)
The U.S. EPA regulates microorganisms and other genetically engineered constructs intended for pesticidal purposes and subject to the Federal

Insecticide Fungicide and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act (FFDCA). The U.S. EPA also regulates certain genetically engineered microorganisms used as biofertilisers.

7.2.1 Other searched databases/sites

Patent Lens [39]
A free public resource for patent system navigation worldwide. No information in addition to other databases was gained from this resource and results are not further mentioned in this report.

Espacenet [22]
A free public resource for patent system navigation worldwide.

8 Appendix 2: Lists of microbial biocontrol products

8.1 List of registered non-GM products worldwide and their active substances

Table 6. List products based on microorganisms (extracted from the Manual of Biocontrol Agents [40])

Active substance	Product
BACTERIA	
Agrobacterium radiobacter K1026	Nogall
Agrobacterium radiobacter K84	Galltrol – A
Aureobasidium pullulans DSM 14940 and DSM 14941	Blossom Protect
Aureobasidium pullulans DSM 14940 and DSM 14941	Boni Protect
Aureobasidium pullulans DSM 14940 and DSM 14941	Botector
Bacillus amyloliquefaciens D747	Double Nickel
Bacillus amyloliquefaciens D747	Double Nickel 55
Bacillus firmus I-1582	Nortica 5% wp
Bacillus firmus I-1582	Poncho/Votivo
Bacillus licheniformis SB3086	Roots EcoGuard (Lebanon Turf)
Bacillus pumilus QST 2808	Ballad Plus
Bacillus pumilus QST 2808	Sonata
Bacillus sphaericus 2362 H5a5b	VectoLex
Bacillus subtilis KTSB	FoliActive
Bacillus subtilis MBI 600	Subtilex NG
Bacillus subtilis QST713	Cease
Bacillus subtilis QST713	Rhapsody
Bacillus subtilis QST713	Serenade ASO
Bacillus subtilis QST713	Serenade Max
Bacillus subtilis QST713	Serenade Soil
Bacillus subtilis subsp. *amyloliquefaciens* FZB24	Taegro
Bacillus thuringiensis aizawai NB200	FlorBac
Chromobacterium subtsugae PRAA4-1	Grandevo
Clavibacter michiganensis subsp. michiganensis bacteriophage	AgriPhage
Pseudomonas chlororaphis MA342	Cedomon; Cerall
Pseudomonas fluorescens A506	BlightBan A506
Pseudomonas spp. DSMZ 13134	Proradix
Pseudomonas syringae ESC 11	Bio-Save 11LP
Pseudomonas syringae pv tomato bacteriophage	AgriPhage
Streptomyces griseoviridis K61	Mycostop
Streptomyces lydicus WYEC 108	Actinovate AG
Xanthomonas campestris pv vesicatoria bacteriophage	AgriPhage

Active substance	Product
ENTOMOPATHOGENIC FUNGI	
Beauveria bassiana ATCC 74040	Naturalis; Racer
Beauveria bassiana GHA	BotaniGard; Botanigard 22WP; Botanigard ES; Eco-Bb; Mycotrol; Mycotrol ES; Mycotrol O
Isaria fumosorosea apopka 97	PreFeRal
Lecanicillium muscarium Ve-6	mycotal
Metarhizium anisopliae BIPESCO 5/F52	Met 52 Granular; Pacer-MA
Metarhizium anisopliae ESF1	*Metarhizium anisopliae* ESF1
Metarhizium anisopliae subsp. *acridum*	Green Guard
Metarhizium anisopliae subsp. *acridum* IMI 330189	Green Muscle
Paecilomyces fumosoroseus Fe9901	NoFly ; NoFly WP
Paecilomyces lilacinus 251	BioAct WG; Melocon
Paecilomyces lilacinus BCP2	PL Gold
Verticillium albo-atrum WCS850	Dutch Trig
FUNGI	
Alternaria destruens 059	Smolder; Smolder G
Ampelomyces quisqualis M-10	AQ 10
Aspergillus flavus AF36	*Aspergillus flavus* strain AF36
Aspergillus flavus NRRL 21882	Alfa-Guard GR
Candida oleophila O	NEXY0101
Chondrostereum purpureum HQ1	Limited or no product currently available
Chondrostereum purpureum PFC 2139	Chontrol Paste; Chontrol Peat Paste
Coniothyrium minitans CON/M/91-08	Contans WG
Cloniostachys (formerly *Gliocladium*) *catenulatum* J1446	Prestop; Prestop Mix
Cloniostachys (formerly *Gliocladium virens* GL-21	SoilGard
Myrothecium verrucaria AARC-0255	DiTera
Phlebiopsis gigantea (several strains)	Rotstop
Pseudozyma flocculosa PF-A22	*Pseudozyma flocculosa* PF-A22
Purpureocillium lilacinus	Biostat
Pythium oligandrum M1	Polyversum
Trichoderma asperellum ICC 012	Bio-Tam
Trichoderma asperellum T25	Tusal
Trichoderma asperellum T34	T34 Biocontrol
Trichoderma asperellum TV1	Xedavir
Trichoderma atroviride I 1237	Esquive WP
Trichoderma atroviride IMI 206040	BINAB TF WP; Binab TF WP
Trichoderma atroviride LC 52	Tenet
Trichoderma atroviride T-11	*Trichoderma atroviride* T-11
Trichoderma gamsii ICC 080	Bio-Tam
Trichoderma hamatum TH382	Incept
Trichoderma harzianum ITEM 908	*Trichoderma harzianum* ITEM 908
Trichoderma harzianum T-22 RIFAI (KRL-AG2	Eco-77; Eco-T; PlantShield HC; RootShield Granules; T-22 HC;

Active substance	**Product**
	Trianum-G; Trianum-P
Trichoderma polysporum IMI 206039	BINAB TF WP; Binab TF WP
Trichoderma viride	Ecosom-TV
MICROSPORIDIUM	
Nosema locustae	NoLo Bait

8.2 List of cancelled approvals outside the EU

Table 7. Overview cancelled approvals outside the EU

Trade name and ID-number	Notification number	Species	New characteristic	site
MPVII Bioinsecticide[2]	?	*Bacillus thuringiensis*	Delta endotoxin of *Bacillus thuringiensis* variety *kurstaki.*	[41]
MVP[3] (for control of caterpillars); M-Trak (for control of Colorado potato beetle),	product cancelled October 15, 2004[6]	*Bacillus thuringiensis*	In the products MVP and M-Trak, the *Pseudomonas fluorescens* cell is killed after it produces the crystal protein	[7]
Raven™ OF bioinsecticide	US EPA 70506-260, cancelled July 21, 2005[6]	*Bacillus thuringiensis*	Antibiotic resistance genes Cry3Bb and 3Aa proteins from *B. thuringiensis* var. *tenebrionis*	[28], pers. comm. C. Wozniak
ProAct, Harp-N-Tek, Mighty Plant, Zolera	EPA Reg. No. 71771-3, 71771-7, 71771-10, 66330-422	harpin αβ protein	Production of harpin αβ protein from *Erwinia amylovora by E. coli*	Pers. comm. C. Wozniak
Mattch bioinsecticide	EPA 55638-17. Cancelled Oct 15-2004[1]	*Pseudomonas fluorescens*		[41], pers. comm. C. Wozniak
Mattch II bioinsecticide	cancelled Oct 24-2004	*Pseudomonas fluorescens*	Cry proteins expressed in *Pseudomonas fluorescens* cells, cells then killed prior to application	[41]
M-Cap[4]	cancelled October 24, 2003[1]	*Pseudomonas fluorescens*	CryIC derived delta endotoxin of *Bacillus thuringiensis* encapsulated in killed *Pseudomonas fluorescens*	[41]
M-Cap[4]	cancelled October 24, 2003[1]	*Pseudomonas fluorescens*	A blend of Cry1A(c) and Cry1C derived delta endotoxins of *Bacillus thuringiensis* encapsulated in killed *Pseudomonas fluorescens*	[41]
M-One Plus[4]	cancelled July 19,	*Pseudomonas*	Delta endotoxin of *Bacillus thuringiensis*	[41]

Trade name and ID-number	Notification number	Species	New characteristic	site
	1995[1]	*fluorescens*	subsp. *san diego* encapsulated in killed *Pseudomonas fluorescens*	
Frostban[3]	product cancelled voluntarily by registrant March 11, 2009,	*Pseudomonas syringae, Ice-mutant,* 742RS	The ice-minus mutant of *Pseudomonas syringae* lacks the gene responsible for ice-nucleating surface protein production. Spraying these bacteria on plant surfaces or fruit reduces frost damage	[41], pers. comm. C. Wozniak
Bio-Trek 22	Registered in 1995 but no current registration at U.S. EPA site	*Trichoderma harzianum* strain 1295-22, Protoplast fusant	Effective in reducing dollar spot, *Pythium*, and brown patch as well as enhancing root growth and increasing plant vigor	
Technical Trypsin Modulating Oostatic Factor (TMOF), insecticide	products cancelled July 21, 2005	trypsin modulating oostatic factor	Trypsin modulating oostatic factor (TMOF), is a 10-amino acid protein (decapeptide) whose genetic coding was isolated from a mosquito and engineered into *Pichia pastoris* yeast	pers. comm. C. Wozniak

1: cancelled for non-payment of maintenance fees
2: Mycogen's MVP product was the first recombinant *Bt*-based MBCA to be registered by the U.S. EPA. Ecogen commercialised several recombinant *Bt* MBCAs. In the table a mismatch between product and producer is very well possible as the sources were not very clear
3: Field-testing of Frostban was the first release of a genetically modified organism into the environment
4: information has been combined from several sources. The information could not be verified and is possibly not correct

9 Appendix 3: Comparison table based on data requirements of Regulation (EC) (1829/2003) on genetically modified food and feed

9.1 Description of the cases

Case 1.
***Pseudomonas/Fusarium*/lettuce/seed coating (Lettuce Guard)**
Biocontrol of *Fusarium oxysporum* in lettuce using *Pseudomonas putida.*

The pathogen
The soil fungus *Fusarium oxysporum* is known to infect a wide range of crops. In lettuce, *F. oxysporum* f.sp. *lactucae* causes lettuce wilt. The disease causes mild stunting to complete collapse of the plants. Diseased plants had severely rotted taproots, from which the fungus can be isolated. *F. oxysporum* is widespread in agricultural soils throughout the world and is commonly isolated from the roots of healthy plants. Most strains are weak parasites that grow only in the root cortex and cause no visible damage to their host plant. However, some strains invade the water-conducting tissue (xylem) and restrict the flow of water and cause wilting.

Mode of action unmodified P. putida
P. putida is a Gram-negative, rod-shaped, saprotrophic soil bacterium. These bacteria are able to colonise all external and internal tissues of plants, including parts used for food or feed.
P. putida has demonstrated biocontrol properties as an effective antagonist of many plant diseases, such as the causal agents of damping-off diseases (e.g. *Pythium* and *Fusarium).* Known modes of action are the production of antifungal components that inhibit pathogens, competition for nutrients, or induced resistance of plants. *P. putida* is also known as a plant growth promoting rhizobacterium (PGPR). PGPRs improve the fitness of host plants. Its mode of action includes the production of siderophores, which have a high affinity for Fe^{3+}. In iron-deficient environments this is a growth stimulation factor for the plant.

Mode of action of the GM-Pseudomonas trait
P. putida is genetically modified with a sensor that detects fusaric acid excreted by the pathogenic fungus *F. oxysporum*. Upon sensing fusaric acid, antifungal components will be produced by the GM *Pseudomonas* strain to suppress *F. oxysporum* infection and protect the lettuce plants. These components are produced by a newly introduced gene.

Type of application/formulation
P. putida is applied as a seed coating.

Compartment of residues
After germination, the Pseudomonas bacteria will colonise the roots and the above-ground plant parts. The GM bacteria can also colonise the lettuce leaves internally, as endophytes.

Case 2.
***Beauveria bassiana*/*Leptinotarsa decemlineata*/ potatoes/granule**
Biocontrol of *Leptinotarsa decemlineata* in potato using *Beauveria bassiana.*

The pest
Larvae of the Colorado potato beetle *Leptinotarsa decemlineata* are an increasing problem in potato crops, because of the damage to the potato crop by their frass. Up to three generations are produced within one season. Full-grown larvae drop from the plants and burrow themselves into the soil to pupate.

The unmodified Beauveria bassiana
Beauveria bassiana is an entomopathogenic fungus that is able to infect and kill insects. Applications of granules with *B. bassiana* will lead to colonisation of the soil bound larvae of the Colorado beetle. The fungus invades the insect's body, usually through the cuticle. After invading the host, the fungus grows throughout the body. Towards the end of mycelium growth SMs are produced which play a role in the death of the larva. Under proper conditions, the fungus will sporulate on the surface of the host cadaver. Sporulation will take place in the soil. The production of secondary inoculum on the host insect may contribute to increased mortality. Another benefit of using *B. bassiana* is overwintering adults may also become infected in the soil.

Mode of action new GM Beauveria trait
Four genes from spiders and scorpion coding for insect toxins are introduced in the genome of *Beauveria*. The modified strain of *Beauveria* is more effective in killing the larvae than the non-gm *Beauveria.*

Type of application
Applications of granules to the soil.

Compartment of residues
After sporulation on the host surface the spores will be able to survive in the soil for a certain period of time. It is not excluded that the GM *Beauveria* will also colonise the potato plant internally, as endophytes.

Case 3.
***Bacillus thuringiensis*/insect larvae/maize/spray application**
Biocontrol of insect larvae in maize using *Bacillus thuringiensis (Bt).*

The pests
The European stem borer *Ostrinia nubilalis* is a moth that causes serious damage to both sweet corn and grain corn. The presence of one to two larvae within a corn stalk is tolerable, but the presence of any larvae within the ear of sweet corn is considered intolerable by commercial growers, and is their major concern.

Dipteran pests such as the ear fly (Diptera, Usectioniidae) is a species that causes increasing problems in maize. Several fly species within this family cause damage to maize plants, especially in sweet cultivars. The principal injury occurs on the developing ear, where they often hollow

out the kernels. Larvae can be found feeding along the entire length of the ear. Yield reductions can reach 100%, with peak levels of injury occurring early in the season.

Bacillus thuringiensis
Bacillus thuringiensis is considered to be a soil bacterium. The life cycle of *Bt* is characterised by two phases which include vegetative cell division and spore development. The vegetative cell is rod-shaped and divides into two uniform daughter cells. Sporulation, on the other hand, involves asymmetric cell division. One part of the cell contains the spore while the other part contains parasporal crystals. The crystals are also called proteinaceous crystals or Insecticidal Crystal Proteins (ICPs) or δ-endotoxins and are considered to be proteins.

Mode of action Bacillus thuringiensis
Bt has to be eaten by insects to cause mortality. The *Bt* toxins dissolve in the high pH insect gut and become active. The crystals then attack the gut cells of the insect, punching holes in the lining. The *Bt* spores spill out of the gut and germinate in the insect causing death within a couple days. Living *Bt* bacteria may also colonise the insect which can contribute to death. It depends on the formulation whether live *Bt* bacteria are present. The formulation may also only contain spores.

Mode of action new GM-Bacillus thuringiensis trait
The genetically modified strain contains a CodY protein which has high insecticidal activity against dipterous insects in addition to its inherent activity against the cotton bollworm *Helicoverpa armigera.* This new trait thus confines an extended host range.

Type of application/formulation
Spray application of spores.

Compartment of residues
Spores including the *Bt* crystals, may survive on the plant, and after harvest, spores may survive on residues of the plant in the soil.
The bacteria also may survive in the soil. Survival on the plant also occurs but is severely limited due to climatic factors and UV light.

9.2 Exercise to uncover gaps, using the three cases

The three cases were submitted to a list of questions, which are indicated in table below. As safety of food/feed treated with GM MBCAs was identified to be a possible gap in the legislation applicable for GM MBCAs, the questions on the three cases will have a strong focus on this issue. The purpose was to predict whether residues of GM MBCAs can remain on or in food/feed products treated with these GM MBCAs and to verify if all the relevant questions in this risk assessment exercise are covered by the existing legislation.

Questions in the table are the same for each case and are grouped under three different headings:
Biocontrol product, the organism, and *the products formed by the organism* in order to address all possible aspects of the fate and survival of the biocontrol product/micro-organism/SMs.

The answers are based on a worst case scenario unless it directly follows from the nature of the organism or the genetic modification that a certain occurrence will not take place. A worst case scenario assumes that in case there is no complete certainty, the chance that the occurrence will take place is 100%.

Table 8. Case 1: Pseudomonas putida on lettuce

Question	**Answer**	**Covered by any Regulation?**
The biocontrol product (new *Pseudomonas components*)		
Are the GM metabolites already present in the formulated product as a consequence of the production method?	Since the new components produced by the GM *Pseudomonas* will be produced only after sensing fusaric acid and fusarium is not present in the medium, the GM metabolite is not present in the product	(EC) 1107/2009 section[1] 2.8
If the former question is answered with yes: After application, do GM metabolites survive in the environment (soil, root, phylloplane)?	Yes, after induction by fusaric acid the antifungal components produced by the pseudomonas strain can be present in the environment for a certain period before they are broken down	(EC) 1107/2009 section[1] 6.1, 7.1 2001/18/EC
The organism *Pseudomonas* (the living bacterium)		
After application, does it survive and spread in the environment (soil, root, phylloplane)?	The bacterium can survive in the rhizosphere and phylloplane and also endophytically	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
After application, in which compartments is reproduction of GM *Pseudomonas* possible?	Reproduction is possible in the rhizosphere of the lettuce plants. Reproduction is also possible on the phylloplane but probably strongly depends on relative humidity and availability of exudates	(EC) 1107/2009 section[1] 7.1 + 2001/18/EC
Does the GM *Pseudomonas* reach the food chain?	Yes, *P. putida* is expected to colonise the phyllosphere. It may also be able to grow endophytically	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
Does GM *Pseudomonas* grow endophytically?	*Pseudomonas putida* strain VM1453 has been identified as an endophyte [25]. It is however not clear whether all strains of *P. putida* are adapted for living *in planta*	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC

Does the GM *Pseudomonas* survive in the edible part of the crop during crop growth, harvest or during processing?	Survival of *P. putida* in the phyllosphere is possible. Bacteria growing endophytically are protected from environmental factors and can be expected to survive for a longer period of time	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
If the former question is answered with yes, is the GM *Pseudomonas* in food/feed toxic or pathogenic to human/animals/non-target organisms?	No, *Pseudomonas putida* is not mentioned in the list of the Regulation (EC) 396/2005 and the newly introduced traits are not expected to give rise to toxicity or pathogenicity for humans, animals and non-target organisms. This should be determined experimentally	(EC) 396/2005
Does the GM *Pseudomonas* have a selective advantage?	It can have a selective advantage since the GM bacterium can survive in the vicinity of *Fusarium* as a result of the genetic modification	2001/18/EC
The products produced by GM *Pseudomonas* (new component)		
Does the GM *Pseudomonas* produce (toxic) GM metabolites as a consequence of the genetic modification) that are different from those of the non-GM *Pseudomonas* strain?	Yes, the GM *Pseudomonas* produces new GM metabolites that are not produced by the non-GM strain. The GM bacterium will produce GM metabolites upon sensing fusaric acid. As the pathogen *F. oxysporum* grows on and in the roots of the lettuce plants, GM metabolites can be produced in the plant and in the rhizosphere	2001/18/EC
Are concentrations of the GM metabolites in or on the treated foodstuffs or feeding stuffs expected to occur in concentrations higher than under natural conditions?	Yes, as these GM metabolites are only produced in the GM *Pseudomonas* they are expected to occur in or on the treated foodstuffs or feeding material in higher concentrations than in the situation of the non-GM *Pseudomonas*	(EC) 1107/2009 Section[1] 2.5; + 2001/18/EC;
Do the GM metabolites formed by the GM	Yes, this is possible. As the pathogen *F. oxysporum* can	(EC) 1107/2009 section[1] 4.2

Pseudomonas reach the edible parts of the food chain?	grow in roots of the lettuce plants, GM metabolites can be produced in the roots, in synergy with the pathogen. Transport of GM metabolites within the plant can occur	+ 2001/18/EC
If the former question is answered with yes, are the GM metabolites persistent in the food/feed? Can consumers be exposed to the residues of GM *Pseudomonas?*	Yes. It is assumed that GM metabolites can be transported into the above-ground plant material. As GM metabolites are not exposed to UV-light, low humidity etc., it is assumed that the GM metabolites remain stable in the plant	(EC) 1107/2009 section[1] 2 + Incidental consumption 2001/18/EC
If the former question is answered with yes: Are the GM metabolites in the food/feed toxic (acute or chronic)?	The GM metabolites are not expected to be toxic for human and animals as the mode of action is specific for fungal pathogens, but this should be determined experimentally	(EC) 1107/2009 section[1] 5 and 6 + Incidental consumption 2001/18/EC

1: The sections mentioned in this table actually refer to Commission Regulation (EU) 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) 1107/2009 placing of plant protection products on the market.

Table 9. Case 2: Beauveria bassiana on potato

Question	**Answer**	**Covered by any Regulation?**
The biocontrol product (contains the *Beauveria* spores)		
Are GM metabolites already present in the formulated product as a consequence of the production method?	The biocontrol product is a granule. The spores in the granule contain low quantities of newly formed components	(EC) 1107/2009 section[1] 2.8
If the former question is answered with yes: After application, do GM metabolites survive in the environment (soil, root, phylloplane)?	The newly formed insect toxins may be stable in the rhizosphere	(EC) 1107/2009 section[1] 6.1, 7.1 + 2001/18/EC
The organism (spores and mycelium are considered to be the organism)		
After application, does it survive in the environment (soil, root, phylloplane)?	*Beauveria* is able to survive saprophytically in the soil but it only sporulates in insect larvae. Initial concentrations after applications eventually return to background levels. Due to reinfestations in suitable hosts *Beauveria* may replenish its volume in the soil and remain present at concentrations higher than the natural background level. *Beauveria* is not known to live in close association with roots and phylloplane	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
After application, in which compartments is reproduction of GM *Beauveria* possible?	Reproduction occurs within insects	(EC) 1107/2009 section[1] 7.1 + 2001/18/EC
Does the GM *Beauveria* reach the food chain?	*Beauveria* grows inside the pest insect. These insects live in the rhizosphere. It depends on the principal point of access into the plant whether *Beauveria* is able to colonise the plant to grow endophytically	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC

Does it grow endophytically?	Yes, some *Beauveria bassiana* strains are known grow endophytically[2]	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
Does GM *Beauveria* survive in the edible part of the crop growth, harvest or during processing?	If *Beauveria* grows endophytically it is expected to survive in the edible part of the crop	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
If the former question is answered with yes, is the GM *Beauveria* in food/feed toxic or pathogenic to human/animals/non-target organisms?	*Beauveria bassiana* is not mentioned in the list of the Regulation (EC) 396/2005, and is therefore not recognised as a human pathogen. The newly introduced traits may give rise to toxicity for humans, animals and non-target organisms. This should be determined experimentally	(EC) 1107/2009 section[1] 5 + (EC) 396/2005 + 2001/18/EC
Does the GM *Beauveria* have a selective advantage?	It does have a selective advantage. The cocktail of toxins is expected to give the organism a selective advantage in the presence of the pathogen	2001/18/EC
Novel metabolites produced by the GM organism		
Does the GM *Beauveria* produce (toxic) SMs that are different from non-GM *Beauveria* strain?	Yes, the GM *Beauveria* produces four scorpion and spider toxins that are not produced by the non-GM strain. These toxins will only be produced inside the host insect	2001/18/EC
Are concentrations of the SMs in or on the treated foodstuffs or feeding stuffs expected to occur in concentrations higher than under natural conditions?	It depends on the ability of this *Beauveria* strain whether it can grow endophytically. If it is also able to produce the toxins in the plant	(EC) 1107/2009; section[1] 2.5 + 2001/18/EC
Do the toxins formed by the GM *Beauveria* reach the edible parts of the food chain?	This is not expected, also not in case of endophytic growth of *Beauveria*[3], since the GM toxins will only be produced after growth in the insect that lives in the rhizosphere. On the other hand, the toxins may reach the edible parts since potatoes are formed in the soil	(EC) 1107/2009 section[1] 4.2 + 2001/18/EC
If the former	The toxins will be formed in the	(EC)

question is answered with yes, are the toxins persistent in the food/feed? Can consumers be exposed to the toxins?	pest insect that lives in the rhizosphere. This is in proximity to the formed potatoes and thus the toxins may reach the potatoes. It is not known how persistent these toxins can be in potatoes, also not after cooking (food) or in pulp (feed)	1107/2009 section[1] 2 + Incidental consumption 2001/18/EC
If the former question is answered with yes: Are the toxins in the food/feed toxic (acute or chronic)?	It is not known if these GM toxins are toxic to humans and animals	(EC) 1107/2009 section[1] 5 and 6 + Incidental consumption 2001/18/EC

1: The sections mentioned in this table actually refer to Commission Regulation (EU) 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) 1107/2009 placing of plant protection products on the market.
2: *Beauveria bassiana* has been isolated from maize and coffee plants [43]. Supposedly, the presence of fungal SMs in the plants cause feeding deterrence or antibiosis in insect pests. It is however not clear whether all *Beauveria* strains have the capacity to grown endophytically.
3: Field experiments have been performed to measure the presence of SMs produced by several entomopathogenic fungi in several types of crops. SMs were not found or were below the detection limit (EU Project RAFBCA).

Case 3. *Bacillus thuringiensis* on maize

The phylloplane of the maize crop is exposed as the application is performed directly on the canopy.
The soil is exposed as part of the application, as the bacteria and the spores will directly fall on the soil or indirectly via dripping off the leaves.

Table 10. Case 3: Bacillus thuringiensis on maize

Question	**Answer**	**Covered by any Regulation?**
The biocontrol product (*Bacillus* and co-formulants)		
Are GM metabolites (Bt toxins) already present in the formulated product as a consequence of the production method?	Probably all formulations contain spores. Therefore, the GM Bt toxin (proteinaceous crystal) is already present in the formulation.	(EC) 1107/2009 section[1] 2.8
If the former question is answered with yes: After application, do GM metabolites	The toxins from *B. thuringiensis* can accumulate in soil and retain insecticidal activity. *Bt* can survive in the soil	(EC) 1107/2009 section[1] 6.1, 7.1 + 2001/18/EC

survive in the environment (soil, root, phylloplane)?	and in the phylloplane but it is considered to be a poor leaf coloniser. The spores are assumed to survive in the soil and in the phylloplane.	
The organism *Bacillus* (the living bacterium)		
After application, does it survive and spread in the environment (soil, root, phylloplane)?	The application is a spray application containing viable spores. Depending on the formulation, living bacteria may also be present. Soil: The soil is considered to be a storage milieu for Bt spores were they remain active for a long period of time. Canopy: *Bt* can survive in the canopy but it is considered to be a poor leaf coloniser, being found mostly as spores in these habitats. Survival is also achieved by recolonization of the canopy after sporulation in cadavers. Roots/rhizosphere: *Bt* can survive in the rhizosphere[1]	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
After application, in which compartments is reproduction of GM *Bacillus* possible?	Reproduction of *Bt* can take place in all compartments of the plant	(EC) 1107/2009 section[1] 7.1 + 2001/18/EC
Does the GM *Bacillus* reach the food chain?	As applications are performed on maize, the spores are present in the crop and may still be present after harvest as spores are able to survive for a considerable period of time.	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
Does GM *Bacillus* grow endophytically?	*Bt* has been shown to grow endophytically[2].	(EC) 1107/2009 section[1] 6.1 + 2001/18/EC
Does the GM *Bacillus* survive in the edible	Food: For human consumption only the	(EC) 1107/2009 section[1] 6.1

part of the crop during crop growth, harvest or during processing?	corn cobs are used. Spores may be present in the cob or on the shielding leaves of the corn cob but these are removed before cooking the corn cobs. Feed: Depending on the type of cattle, corn cobs and/or the rest of the plant are used for feed. Cattle may be exposed to *Bt*, or its spores.	+ 2001/18/EC
If the former question is answered with yes, is the presence of the GM microbial in food/feed pathogenic/toxic to human/animals/non-target organisms?	*Bacillus thuringiensis* is not mentioned in the list of the Regulation (EC) 396/2005 and there are no indications for pathogenicity found in the literature. The newly introduced trait may potentially lead to toxicity of the GM *Bacillus* to non-target organism, this should be tested.	Regulation (EC) 396/2005
Does the GM *Bacillus* have a selective advantage?	It may have a selective advantage due to the toxin production.	2001/18/EC
The products produced by the GM *Bacillus* (the crystal)		
Does the GM *Bacillus* produce toxic crystals that are different from non-GM *Bacillus* strain?	Yes, the GM toxins are different with respect to their host range. These toxins affect Dipteran species.	2001/18/EC
Are concentrations of the SMs in or on the treated foodstuffs or feeding stuffs expected to occur in concentrations higher than under natural conditions?	Yes, Bt toxins are not produced by the non-GM *Bacillus.*	(EC) 1107/2009 Section[1] 2.5 + 2001/18/EC
Do the GM proteins formed by the GM *Bacillus* reach the edible parts of the food chain?	Food: Yes, the GM metabolites may be formed on or in the edible part of the food chain Feed: The GM metabolites	(EC) 1107/2009; section[1] 4.2 + 2001/18/EC

	may be present on or in the edible parts when used as feed.	
If the former question is answered with yes, are the toxins persistent in the food/feed? Can consumers be exposed to the residues of GM *Bacillus?*	Toxins may persist on the surface or in the food/feed parts of the crop, therefore humans may be exposed by food. Animals fed with whole shredded corncobs may be exposed.	(EC) 1107/2009 section[1] 2 + Incidental consumption 2001/18
If the former question is answered with yes: Are the toxins s in the food/feed toxic (acute or chronic)?	The GM Bt toxins are not toxic for humans and animals as the mode of action is specifically targeted against Diptera.	(EC) 1107/2009 section[1] 5 and 6 + Incidental consumption 2001/18

1: The sections mentioned in this table actually refer to Commission Regulation (EU) 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) 1107/2009 placing of plant protection products on the market. Refer to Appendix 10.2 for the data requirements mentioned in Commission Regulation (EU) 283/2013.
2: Endophytic growth has been demonstrated in several different crops [45]. Possible routes of entry are the roots, the epidermis and the stomata. Epiphytes colonizing plant tissues, can reach the seeds systemically and transmission then occurs via the seeds (references in [44]).

Based on this exercise, two main conclusions can be drawn with regard to the food and feed safety:

1. a GM MBCA can persist as a residue on or in the food/feed product and can potentially affect the food/feed safety of the product;
2. Novel Metabolite(s) produced as a consequence of the genetic modification can persist in or on the food/feed product and can potentially affect the food/feed safety of the product.

The tables also show that the most important aspects of food and feed safety with respect to the GM MBCA and its metabolites are addressed in the applicable legislation.

10 Appendix 4: Comparison table based on data requirements of Regulation (EC) (1829/2003) on genetically modified food and feed

In Table 11 it is checked whether the data requirements of Regulation (EC) 1829/2003[3] on genetically modified food and feed are covered by Directive 2001/18/EC and Regulation (EC) 1107/2009. This exercise will reveal potential gaps in the risk assessment of GM MBCAs.

Reading guide to Table 11:

1. Regulation (EC) 1829/2003 deals with the risk assessment of GM food/feed. In this exercise not the plant but the GM MBCA will be evaluated. For this reason the 'plant' which is normally the subject of the evaluation in Regulation (EC) 1829/2003 (data requirements in the left column) should be read as 'GM MBCA'.
2. In sections 1.4 on Toxicology and 1.5 on Allergenicity. Regulation (EC) 1829/2003 would normally assess the toxicological and allergenicity effects of the GM food/feed item. In this exercise the food/feed containing residues of GM MBCAs is assessed in these particular sections.
3. In cases that the data requirement is not covered in any of the two legislations (Directive 2001/18/EC nor Regulation (EC) 1107/2009) the rows are shaded in orange.

[3] The data requirements are set in Commission implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) 1829/2003 of the European Parliament and of the Council. For matter of convenience Regulation (EC) 1829/2003 is referred to.
The rules laid down in this implementing Regulation specify the general requirements for the presentation and preparation of applications under Regulation (EC) 1829/2003. It specifies the requirements to provide general and scientific information, including methods for detection, and identification, as well as reference material so as to ensure that applications comply with the conditions laid down in Articles 5, 17 and 30 of Regulation (EC) 1829/2003.

Table 11. Comparison table

Data requirements Regulation (EC) 1829/2003		**Covered by Directive 2001/18/EC?**	**Covered by Regulation (EC) 1107/2009?**
ANNEX II	SCIENTIFIC REQUIREMENTS FOR THE RISK ASSESSMENT OF GM FOOD AND FEED		
1	HAZARD IDENTIFICATION AND CHARACTERISATION		
1.1	**Information relating to the recipient or (where appropriate) parental plants**		
	(a) Complete name: (i) family name; (ii) genus; (iii) species; (iv) subspecies; (v) cultivar, breeding line; (vi) common name.	yes, for (v) (= the cultivar or breeding line) this would be the strain	yes, for (v) (= the cultivar or breeding line) this would be the strain
	(b) Geographical distribution and cultivation of the plant within the Union;	yes, only geographical distribution of the MBCA	yes, only geographical distribution of the MBCA
	(c) Information on the recipient or parental plants relevant to their safety, including any known toxicity or allergenicity;	yes	History of safe use of the micro-organism (strain relatives) is taken into account (section 2.1.)
	(d) Data on the past and present use of the recipient plant[4], such as history of safe use for consumption as food or feed, including information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant normal role in the diet (for example, which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).	partly (less thorough; only incidental consumption) The history of safe use of the non-GM microorganism is taken into account, but only for incidental consumption/intake	History of safe use of the micro-organism (strain relatives) is taken into account (section 2.1.)
1.2	**Molecular characterization**		
1.2.1	Information relating to the genetic modification	yes	no
1.2.1.1	Description of the methods used for the genetic modification	yes	no

[4] Recipient plant is the plant into which the new gene has been inserted.

Data requirements Regulation (EC) 1829/2003		Covered by Directive 2001/18/EC?	Covered by Regulation (EC) 1107/2009?
1.2.1.2	Nature and source of vector used	yes	no
1.2.1.3	Source of nucleic acid(s) used for transformation, size and intended function of each constituent fragment of the region intended for insertion	yes	no
1.2.2	Information relating to the genetically modified plant	yes	no, the whole GM MBCA will be assessed. There is no specific focus on the genetic modification
1.2.2.1	General description of the trait(s) and characteristics which have been introduced or modified	yes	no
1.2.2.2	Information on the sequences actually inserted/deleted	yes	no
1.2.2.3	Information on the expression of the insert(s)	yes	no
1.2.2.4	Genetic stability of the insert and phenotypic stability of the genetically modified plant	yes	no, only genetic stability of the GM MBCA itself
1.2.2.5	Potential risk associated with horizontal gene transfer	yes	yes[5]
1.3	**Comparative analysis**		
1.3.1	Choice of the conventional counterpart and additional comparators	yes, in comparison with non-GM line	no, there is no comparison to a non-GM MBCA or other comparators
1.3.2	Experimental design and statistical analysis of data from field trials for comparative analysis	partly (in comparison with non-GM line), only for the agronomic and phenotypic characterization, see 1.3.5.	no
1.3.2.1	Description of the protocols for the experimental design	no	no
1.3.2.2	Statistical analysis	no	no
1.3.3	Selection of material and compounds for analysis	no	no
1.3.4	Comparative analysis of composition	no	no
1.3.5	Comparative analysis of agronomic and phenotypic	yes	no

[5] Uniform principles in Commission Regulation (EU) No 546/2011 say under section 2.2.1.2 that information on the genetic stability of the micro-organism under the environmental conditions of proposed use must be evaluated, as well as information on the micro-organism's capacity to transfer genetic material to other organisms and information on the stability of encoded traits. The micro-organism's capacity to transfer genetic material to other organisms is also referred to in section 2.7 of (EU) 283/2013. It is not specified whether specific genes or all genes are investigated.

Data requirements Regulation (EC) 1829/2003		Covered by Directive 2001/18/EC?	Covered by Regulation (EC) 1107/2009?
	characteristics		
1.3.6	Effects of processing	no	no
1.4	**Toxicology**		
1.4.1	Testing of newly expressed proteins	yes, unless GM MBCA cannot be detected	no
1.4.2	Testing of new constituents other than proteins	yes, unless GM MBCA cannot be detected	no
1.4.3	Information on altered levels of food and feed constituents	no	no
1.4.4	Testing of the whole genetically modified food or feed	no	no
1.4.4.1	90-day feeding study in rodents with whole genetically modified food/feed	no	no
1.4.4.2	Animal studies with respect to reproductive and developmental toxicity testing	no	no
1.4.4.3	Other animal studies to examine the safety and the characteristics of genetically modified food and feed	no	no
1.4.4.4	Interpretation of relevance of animal studies	no	no
1.5	**Allergenicity**		
1.5.1	Assessment of allergenicity of the newly expressed protein	yes, unless GM MBCA cannot be detected	no
1.5.2	Assessment of allergenicity of the whole genetically modified plant	yes, unless GM MBCA cannot be detected	no
1.5.3	Adjuvanticity	no	no
1.6	**Nutritional assessment**		
1.6.1	Nutritional assessment of the genetically modified food	no	no
1.6.2	Nutritional assessment of the genetically modified feed	no	no

The following paragraphs discuss the potential gaps identified in Table 11.

Section number 1.1. Information relating to the recipient or (where appropriate) parental organism
In Regulation (EC) 1829/2003 data are requested on the past and present use of the recipient plant, such as history of safe use for consumption as food or feed, including information on how the plant is typically cultivated, transported and stored. For this exercise the past and present use of the MBCA is considered instead of the plant, such as the history of safe use of the non-GM-MBCA, including information on how this MBCA is typically applied. Information on the history of safe use in case of food/feed use of the MBCA is taken into account under

Directive 2001/18/EC with respect to incidental consumption. Under Regulation (EC) 1107/2009 history of safe use of the will be taken into consideration. This may be the non-GM MBCA or a strain relative.

It is concluded that there is no gap for information relating to the recipient or parental plant.

Section number 1.2. Molecular characterisation
Molecular characterisation of het GM MBCA is fully covered by Directive 2001/18/EC.

It is concluded that there is no gap for the data requirement of molecular characterisation.

Section number 1.3. Comparative analysis
Under the Food and feed Regulation (EC) 1829/2003 the results of the comparative analysis of a GM and non-GM plant (in our case, the comparison between a GM and non-GM MBCA) differ under various conditions. Through this comparative analysis potential (un)intended effects of the genetic modification are studied.
Under Directive 2001/18/EC the possible adverse effects of the GM MBCA on the ecosystem are evaluated in comparison to the non-GM MBCA. If these organisms are present in or on food/feed, effects of incidental consumption/intake of any remaining GM MBCA will be assessed in comparison to that of the non-GM MBCA. Under Regulation (EC) 1107/2009 the history of safe use is considered.
Compositional analysis of the GM MBCA treated food/feed products is not performed under Directive 2001/18 or Regulation (EC) 1107/2009. In the exercise with the three cases it concluded that residues of GM MBCAs could remain on/in the food /feed products and on a case-by-case basis could result in a change in composition of the food/feed.

It is concluded that there is a gap in the risk assessment with respect to the compositional analysis of food/feed parts that have been treated with GM MBCAs.

Section number 1.4. Toxicology
Under the Food and feed Regulation (EC) 1829/2003 it is, among others, assessed whether the introduced sequences may be toxic by using bioinformatics. The sequences of the newly inserted DNA, its bordering regions (junction between insert and genomic region) and of the newly expressed proteins are determined and compared to sequences of known toxins or if endogenous genes are disrupted. In case no similarity to known toxins is found, and no known endogenous genes are disrupted, no further data are required. If there are indications for toxicity, further tests have to be supplied. Under Directive 2001/18/EC this is assessed in the same way, but only for the GM MBCA itself and for its novel metabolites, but not for the food/feed. Under the Food and feed Regulation (EC) 1829/2003 also a 90 day feeding trial with whole food/feed is mandatory. This is not the case under Directive 2001/18/EC or Regulation (EC) 1107/2009.
Under Regulation (EC) 1107/2009 the potential toxicity of the GM MBCA, but not of the food/feed treated with these micro-organisms MBCA, is evaluated for both environment and human toxicology in consideration

of the mode of action of the GM MBCA. If it can be justified from the existing background information (strain relatives) and results of the toxicity tests, that there are no indications that the GM MBCA can produce toxins that are relevant to the environment and humans, then it is decided to refrain from further questions. Thus, it is accepted that small quantities of SMs are produced locally, *in vivo*, upon contact of the GM MBCA and its anticipated pathogen/host. This data requirement can be dealt with in a so called waiver (data requirement is waived with a science based argumentation).
Under Regulation (EC) 1107/2009 tests with rats need to be performed. These tests are performed with the fermentation liquid in which SMs can be present as a residue from the fermentation procedure.
From this exercise it can be concluded that potential toxicity of the GM MBCA is covered in the risk assessment under Directive 2001/18 and Regulation (EC) 1107/2009, but not the potential toxicity of the whole food/feed that may contain residues of GM MBCAs.
As was mentioned before, under the Food and feed Regulation (EC) 1829/2003 a 90 day feeding trial with whole food/feed is mandatory. However, in practice these tests are considered to be too insensitive to be of any use.

It is concluded that there is no gap in the risk assessment with relation to toxicology of the GM MBCA itself. However, a gap in the risk assessment may exist with respect to potential toxicity of the food/feed treated with the GM MBCAs.

Section number 1.5. Allergenicity
Under the Food and feed Regulation (EC) 1829/2003 it is assessed whether the introduced sequences may be allergenic by using bioinformatics. It is assessed whether the introduced sequences can lead to allergenicity of the food/feed product by using bioinformatics. The sequences of the newly inserted DNA, its bordering regions (junction between insert and genomic DNA) and of the newly expressed proteins are determined and compared to sequences of known allergens. In case there is no similarity to known allergens, no further data are required. If there are indications for allergenicity, further tests have to be supplied. This is the same under Directive 2001/18/EC, but only for the GM MBCA and for its novel metabolites, but not for the food/feed. Under the Food and feed Regulation (EC) 1829/2003 are no mandatory animal studies to test for allergenicity.
Under Regulation (EC) 1107/2009 allergenicity to the whole GM MBCA and all its metabolites is evaluated using relevant clinical observations or animal studies, bioinformatic studies are not performed.

It is concluded that there seems to be no gap in the risk assessment with relation to the allergenicity of the GM MBCA itself. However, a gap in the risk assessment may exist with respect to potential allergenicity of the food/feed treated with the GM MBCAs toxicity.

Section number 1.6. Nutritional assessment
Und the Food and feed Regulation 1829/2003, nutritional assessment of whole food/feed is required. However, the assessment of nutritional value of the food/feed does not give any information about the safety of the food/feed. As it does not contribute to the risk assessment this

study is no longer requested in practice. Nutritional assessment is not covered under Directive 2001/18/EC and Regulation (EC) 1107/2009.

It is concluded that there is no a data gap with respect to the nutritional value of the food/feed.

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