

## MINI REVIEW

# Genetically modified microbial symbionts as arthropod pest controllers: risk assessment through the European legislations

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**Abbreviations list:** EC, European Commission; ECHA, European Chemicals Agency; EFSA, European Food Safety Authority; ERA, environmental risk assessment; EU, European Union; FDA, Food and Drug Administration; GMM, genetically modified micro-organism; GMO, genetically modified organism; MS, Member State (of the EU); PPP, plant protection product; RA, risk assessment.

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**Introduction**

Since the earliest studies at the beginning of the last century and the pioneering work of Buchner (1965), symbiosis between insects and bacteria (and to a lesser extent, fungi) has attracted the attention of biologists who have strengthened our knowledge about the nature of such associations and the ecological and evolutionary relationships between them (Beard et al. 1998; Gibson and Hunter 2010; Gosalbes et al. 2010). A large proportion of insects is expected to

**Abstract**

The genetic modification and applied use of microbial symbionts have been identified as novel tools to protect beneficial insects such as pollinators or parasitoids or to fight insects that constitute pests or are vectors of infectious diseases. The deliberate release of insect pest or disease vector control products containing genetically modified micro-organisms (GMMs) can raise questions about health and environmental safety. Different national and international authorities have established legal requirements to ensure the safe use of conventional pesticides and insecticides as well as GMMs. A key requirement is to conduct a scientific risk assessment to determine whether the product is safe to be placed in the market. In this study, we address the legal framework, the regulatory requirements, and the criteria for the environmental risk assessment of GM symbionts that currently apply within the European Union.

carry bacterial symbionts (Chaves et al. 2009). The spectrum of these relationships is wide and the degree of dependency between the symbiont and the host can range from obligate mutualism to transient colonisations of micro-organisms that are able to exist also in the absence of the insect. Host specificity also varies greatly among non-obligate symbionts. The location and biological function of the symbionts vary from colonisation inside or outside of the host cells, and transmission may occur vertically (from the mother to the eggs) or horizontally (from

insect to insect by, e.g. mating or coprophagy) (Marzorati et al. 2006). Symbionts usually exert beneficial effects to their hosts, including the provision of essential nutrients (mostly in the case of obligate mutualists), enzymes for degrading certain carbon sources, resistance to abiotic stress or protection against parasites (Buchner 1965). However, the presence of microbial symbionts can sometimes also be considered negative for the insect. An example case is colonization by *Wolbachia* sp., a bacterium that modifies the reproduction and fitness of its host.

The genetic modification and applied use of microbial symbionts have been identified as novel tools to fight insects that constitute pests or are vectors of infectious diseases. Microbial symbionts could also be used to protect beneficial insects such as pollinators or parasitoids. The precise engineering of the genome of microbial symbionts can potentially lead to altered host fitness or physiological traits that reduce or eliminate their detrimental effects. This approach is termed paratransgenesis and can be used in several ways: for instance, to control the proliferation of insect pests (e.g. crop pests) or to eliminate the capacity of an insect to act as a vector and transmit pathogenic diseases. Several technological barriers limit the current development and application of paratransgenesis. Key challenges include the difficulty to culture and transform many symbiont micro-organisms and to precisely describe its functional context in insect host biology and ecology. One of the most advanced examples of paratransgenesis is the genetic modification of *Rhodococcus rhodnii*, a bacterium present in the reduviid bug *Rhodnius prolixus* that makes it unable to transmit the pathogen *Trypanosoma cruzi* that causes Chagas disease (Durvasula et al. 1997). Other identified targets for paratransgenesis include *Alcaligenes*, a bacterial gut symbiont of the sharpshooter *Homalodisca coagulata* which is the vector of Pierce's crop disease (Bextine et al. 2004), and *Asaia* sp., a bacterial symbiont discovered in *Anopheles stephensi*, one of the vectors of malaria (Damiani et al. 2010).

The commercialisation of an insect pest control product consisting of or containing genetically modified micro-organisms (GMMs) will raise public questions about safety extending beyond the environmental considerations normally taken into account for conventional pest control products. A range of safety concerns are likely to be expressed, as currently seen in the European Union (EU) for genetically modified plants. To protect citizens against real or perceived risks associated with, on one hand, the use of pesticides and insecticides, and on the other hand, the

release of genetically modified organisms (GMOs), different national and international authorities have established legal requirements for the safety of these products. Here we address the legal requirements in matter of environmental safety that currently apply within the EU for this kind of products, focusing on the criteria for the environmental risk assessment of genetically modified micro-organisms and the implications that it can have for the case of paratransgenesis.

## Regulatory Framework

The EU has three legal mechanisms in place to regulate the release and marketing of GMMs, crop pesticides and insecticides, respectively. These are Directive 2001/18/EC on the deliberate release into the environment of GMOs (EC 2001, herein referred to as the 'GMO Directive'), Regulation (EC) No 1107/2009 concerning the placing of plant protection products (PPPs) on the market (EC 2009a, the 'PPP Regulation') and Directive 98/8/EC concerning the placing of biocidal products on the market (EC 1998, the 'biocide Directive')<sup>1</sup>. The latter is currently in revision. The first Directive concerns the environmental release of all types of GMOs, including GMMs. However, although they are clearly within the scope of the Directive, GMMs are not addressed at the same level of detail as GM higher plants. The second Regulation refers to PPPs as several kinds of substances, among them those 'protecting plants or plant products against all harmful organisms or preventing the action of such organisms' that include pesticides against arthropods. The definition of PPP also includes other products such as herbicides or growth promoters, which are therefore also under the scope of the Regulation. Other insecticides are covered by the biocide Directive, whose scope includes substances 'intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism', but excludes the products covered by the PPP regulation.

Although the legislations mentioned above have been produced independently, they share some essential principles and common approaches. All

<sup>1</sup>In the EU legislative system, a Directive is a legislative act whose objectives must be achieved through the implementation of national legislations developed independently by each Member State. In contrast, a Regulation sets out the means to reach its own objective and does not require any implementing measures at the national level.

three of them require that any product under their respective scopes must gain an approval by the European Commission (EC) or the Member States (MS) (depending on the case) before being placed in the market. To be approved, the product must not be harmful for humans, animals or the environment. The mechanism used to identify and evaluate possible harm is, in all cases, an environmental risk assessment (ERA). The ERA must cover all relevant aspects, from a full characterisation of the product to an estimation of possible long-term, unintended effects. To enable the risk assessment, applicants (i.e. those who intend to release or market the product) must submit a complete dossier that compiles all the scientific and technical data (including experimental results from laboratory and field studies) relevant to characterise the risks. Thus, the information provided in the dossier and the ERA will on a case-by-case basis determine the harmlessness of the product. The structure of the applications (called 'notifications' in the case of the GMO Directive) differs according to the legislation's requirements. The information in each application includes legal and administrative requirements such as the identification of the applicant, description of intended uses, conditions for placing into the market, labelling aspects or compliance with other relevant legislations. In all cases, the major part of the application consists of the technical dossier and the risk assessment conducted by the applicant. The competent authorities of MSs or the scientific panels of the European Food Safety Authority (EFSA) subsequently undertake an independent re-evaluation of the information and ERA submitted by the applicant.

Besides these shared approaches, there are aspects specific to each of the three legislations that warrant mentioning:

*GMO Directive:* The Directive does not specifically address the information required for the environmental release of micro-organisms. The requirements described in the annexes of the Directive are divided in two sections: for higher plants or for organisms other than higher plants (which include micro-organisms). However, it considers two types of deliberate releases: those for placing the product in the market and those intended for any other purpose. The latter refers to releases of GMOs in field trials. Field trials are normally carried out before commercialisation of a product, to test the efficacy of the product and collect experimental data for estimation of possible harm. Data from field trials are often necessary for the preparation of the technical dossier.

According to the Directive, any field trial involving GMOs must be subjected to an authorisation process conducted under the same terms as that for marketing. However, for marketing authorisations the Directive does not generally require that the data presented in the dossier is obtained from field trials made in the EU. Hence, two authorisations would be needed only in the case when both the field trials are conducted and the product is marketed in the EU.

*PPP Regulation and biocide Directive:* Both legislations define the specific information to be provided for products of microbial origin. In contrast to the GMO Directive, they do not request specific permits for field trials, but distinguish between the active substance and the product that will be placed into the market. The product includes the active substance (that exerts the desired effect) and any other substances included in the formulation that is, in the end, commercialised. According to the legislations, the active substance must first be assessed for safety and, if considered safe, included in a list of authorised substances. Only then, the product carrying the authorised substance should be assessed and, if safe, approved. Therefore, applicants must submit two dossiers: one for the active substance, and subsequently another for the whole product to be marketed. The active substance can for instance be a GMM. The final product would then depend on how the GMM is intended to be released/commercialised. For example, the final product might then be a feeding system installed in the field that the target insect would feed on and become colonised by the GMM. Alternatively, the product may be an already GMM colonised host insect that can be a source for further dissemination of the active substance.

Applications (for GMOs, PPPs and/or biocides) including the technical dossier and the completed ERA must be submitted to the MS in which the product is intended to be released. This MS is then in charge of evaluating the provided documentation and can request from the applicant any further scientific information that it deems necessary for the safety evaluation. Subsequently, the MS will assemble a report in which it will reason whether the product can be authorised or not. Other MSs can make comments on the report according to their own expert evaluation of the ERA. In case of disagreements between MSs, the European bodies will help in the arbitration. In case of GMOs and PPPs, the EFSA provides an independent and science-based evaluation of the dossier. EFSA is also empowered to ask the applicant for further information as

needed. The role of EFSA varies according to product type. For GMMs, it is limited to the cases of discrepancies between different MSs. If disagreement on safety occurs, EFSA conducts an independent ERA of the GMO, taking into account all comments from the MSs, and publishes an opinion on the safety of the product. Then, and taking into account this opinion, the MSs must decide on authorisation for the release of the GMO. For PPPs, EFSA assesses and concludes on the safety of the active substance in all cases. However, EFSA has no role in the assessment of the final product, which is the responsibility of the MSs where the product is to be marketed. In the case of biocides, the European Chemicals Agency (ECHA) will assess the active substance. Once EFSA or ECHA has issued its conclusion, the European Commission decides to approve or not the active substance. PPPs and biocides containing approved active substances must be further approved at MS level prior to commercialisation.

Symbiont GMMs to be used as tools for pest or disease control would fall under two of these legislations simultaneously. In case of a crop pest control system [e.g. GM *Enterobacter* intended to control populations of pink bollworm (Kuzina et al. 2002)], both the PPP Regulation and the GMO Directive would apply. However, a disease vector control system such as a recombinant *Wolbachia* engineered to prevent mosquito-vector infections would fall under the GMO and the biocide Directives. That would be also the case when the symbiont is engineered to impair transmission of pathogens without killing the host (e.g. a GM *Wolbachia* which shortens the mosquito life span so that a virus life cycle cannot be completed, and hence, the virus transmission frequency is decreased). In the examples above, two different approvals (after evaluation of their respective technical dossiers) would be needed because the legislations operate independently. For the time being, no simplified procedure exists that would allow a unified application. However, the risk assessment and approval processes should not be seen fully independently, as there is some overlap in data requirements. Indeed, the PPP Regulation states that, when the active substance is of microbial origin and has been genetically modified, the technical dossier submitted within the GMO notification should also be included in the application for the active substance. Moreover, the GMM in question should have been authorised under the GMO Directive before any final product in which it is included can be approved. The same approach is foreseen in the revision of the biocide Directive.

## Principles of GMM Risk Assessment

A number of risk assessment principles for GMOs have been developed after various international negotiations (Codex Alimentarius 2004). In the EU, the deliberate release of GMMs is, as explained above, subject to an application for authorisation which requires an ERA. It is the responsibility of the applicant to conduct the ERA and to develop a monitoring plan. The ERA aims to identify and evaluate any adverse effect of the GMM, and should consider direct, indirect and cumulative long-term effects. It must be scientifically sound, done on a case-by-case basis and in accordance with the precautionary principle. The ERA should also be updated when new information is available on the environmental effects of the GMM. A comparative approach is preferred when an unmodified counterpart is available. It is worthwhile to recognize that the ERA of GMMs is risk-oriented and does not include data required for a broader risk-benefit analysis. Data related to potential product benefits are vital to the applicants own marketing strategies and risk communication. However, no standardised approaches exist for the production of data demonstrating direct or indirect product benefits.

Here, we present the key Principles of risk assessment as outlined in Annex II of Directive 2001/18/EC and supplemented by Commission Decision 2002/623/EC (EC 2002a).

## Data Requirements for Risk assessment

Directive 2001/18/EC outlines the data requirements and scientific approaches to conduct RA for the deliberate release of GMOs into the environment. Annex IIIA of the Directive provides a detailed specification of the types of experimental data necessary for the RA. The key scientific data to be produced and presented in the notification can be divided into four categories: (i) data demonstrating a precise understanding of the genetic modification and the GMM itself; (ii) data describing the release method and a necessary knowledge of the receiving environment; (iii) data outlining any interactions (intended or unintended) between the GMM and the environment; (iv) validated and detailed protocols for monitoring and control of the GMM after release. Below we briefly present some of the data requirements and discuss them in the context of the scope of this work (for a complete set of requirements, see Directive 2001/18/EC with annexes and updates as well as EFSA 2006). Some of these requirements and the

principles on which they are based would also be valid for an eventual risk assessment of natural micro-organisms to be released in the environment (e.g. non-recombinant microbial symbionts for pest control). However, such micro-organisms would not need an authorisation under the GMO Directive and therefore are out of the scope of this work.

#### Data on the gene donor, the genetic modification and the GMM

The major biological properties of the donor organism should be described including the properties of the genetic material derived from it, and used to engineer the DNA inserted in the recipient micro-organism (i.e. the transgene[s]). The characteristics of the recipient species should be described including its taxonomic classification, relevant physiological and ecological traits, any pathological properties, as well as its normal habitat and environmental distribution. Two key challenges to the accurate description of the recipient micro-organisms in the ERA are that (i) supportive information may not be available in the scientific literature because many (endo)symbionts have only recently been described and their host interactions are not fully understood and (ii) bacterial populations of the same species are often genetically heterogeneous (Cohan 2004; Chouaia et al. 2010). It can therefore be challenging to describe the genetic coherence and population structure of the recipient species, its host specificity and environmental distribution.

The dossier must also include a detailed description on how the genetic modification was made (including the methods for transformation and selection) and subsequently characterised at the molecular level (structure of the introduced DNA). The characteristics of any vector used in the GM process should be provided including its nature, source, sequence, mobilisation frequency and host range. These data are essential for the ERA in case the vector or any parts of it remains in the GMM, a possibility that should be tested and the relevant data provided. Particular attention should be paid to the possible presence of any vector-related antibiotic resistance gene. A clear-cut demonstration of genetic stability and absence of mobility of the transgene(s) is desirable and thus, such transgene(s) should preferably be inserted/modified in the host chromosome and not be present on extrachromosomal plasmids or other mobile genetic elements.

Another essential part of the dossier is the phenotypic characterisation of the GMM including a

detailed description of the new phenotypic traits (e.g. expression level and variability of the introduced genes in relevant environments). The biological activity of any newly expressed proteins should be known and sufficient data should be available to demonstrate the intended effects and to assess unintended effects on human, animal and plant health (e.g. direct toxicity, allergenicity or altered host pathogenicity).

As indicated above, extensive scientific data sets are required to understand the biology of the unmodified micro-organisms and its GM counterpart. A substantial amount of the data required is not directly linked to the genetic modification process or outcome, but to the basic understanding of the biology of the recipient organism itself. Thus, GM approaches in recipient species with well-known biology, an established history of safe use, and familiarity would clearly benefit the science-based ERA.

#### Conditions of release and the GMM receiving environments

Detailed information on the nature of the intended releases and release methods/strategies must be provided. Moreover, a detailed description of the receiving environments (e.g. geographic location, exposed ecosystems, prevalence and distribution of affected target and non-target species, etc.) is necessary for the ERA. The ERA of GM symbionts will often be extensive as pest insects normally feed from several sources, travel over large distances (e.g. locusts) and have a plethora of interactions with other organisms (predators, other symbionts, etc.). Strategies enabling the removal of the GMM from the receiving environment should be described. This information is necessary to demonstrate a precise understanding of the exposure dynamics and control of the release in the receiving environments, as well as to exclude any negative effects on non-target organisms, including humans.

#### GMM and its environmental interactions

A key consideration made in environmental risk assessment is how the genetic engineering process has or has not affected the survival, multiplication and dissemination potential of the micro-organism. Thus, it is important that sufficient scientific information is available for the ERA to understand the host range and key population parameters of the GMM. Such data should also be robust in relation to

variation in abiotic and biotic environmental variables.

Beyond the assessment for unintended population expansion of the GMM itself, the ERA should also address the genetic containment and stability of the transgenes and their potential for unintended transfer to other organisms present in the same environment (i.e. via horizontal gene transfer, Thomas and Nielsen 2005). The potential for horizontal gene transfer and stabilisation of the transgenes (as well as unintended population expansion of the GMM) rest on the potential selective advantage conferred by the transgenes on its host. Thus, an important consideration is to identify and understand the implications of the selective advantage or disadvantage of the trait conferred by the genetic modification on host relative or absolute fitness. The data requirements on specific interactions with target and non-target organisms can be extensive as the GMM symbiont is likely to be released in open environments. Thus, a challenge to the evaluation of the environmental effects of the GMM is the lack of baseline data that can assist in the description of the biology of the receiving environment to a level where precise predictions can be made about the effects of introducing a particular GMM.

#### Monitoring and control of the GMM

Council Decision 2002/811/EC (EC 2002b) provides guidance notes on the environmental monitoring plan, and supplements Annex VII of Directive 2001/18/EC). Validated protocols for monitoring of the GMM after release should be provided. The protocols must be able to trace the recombinant DNA and identify the GMM. The detection protocol must therefore target the DNA insertion 'event' to be specific for the GMM. Moreover, detailed plans for post-release monitoring must be presented as well as a description of how such information will be assembled and processed. The rationale behind the monitoring plan is either (i) to provide a protocol for case-specific collection of specific data about risk/uncertainty issues that were raised in the ERA but not fully solved or (ii) to provide a protocol for general surveillance to identify or exclude the occurrence of unintended effects (e.g. indirect, cumulative, delayed) not previously identified in the ERA. Thus, in both cases, the monitoring plan will contribute to confirm assumptions, reduce uncertainty and increase knowledge of the GMM and its environmental interactions. The monitoring plan should, on a case-by-case basis, describe a methodological

approach to the systematic observation of relevant biological properties of the exposed environment. The plan must also identify who is responsible for the monitoring and reporting to the competent authority.

A description should be made if there are methods available for confining and controlling the GMM release and dissemination to the area of intended release. Finally, emergency response plans should be described in case of unintended/unexpected dissemination of the GMM. Such plans should contain consideration of methods that will control spread (treatment of affected areas), protection measures or means of recall and eradication. This latter ERA component seems essential to enable an efficient risk communication of product performance and control in a public perception perspective.

As highlighted above, the ERA of GMM symbionts requires the availability and analyses of an extensive set of biological data. It is the responsibility of the applicant to identify, produce, assemble, assess and present such data in the notification. The data needed for the ERA will be derived from various sources. The applicant will have to produce a substantial part of the product-specific data through laboratory analyses and experimentation. In addition, all relevant studies available in the scientific literature to describe, for example, the biological properties of the recipient organisms and its environment (baseline data) must also be provided and taken into account for the ERA.

Because of differences in availability, relevance and quality of the baseline data collected from external sources, a number of inferences and assumptions will likely have to be made. The reasoning behind these assumptions may or may not be shared by MS or EFSA (ECHA in case of biocidal products) in subsequent evaluations. Some risk assessment issues that may rest on assumptions are: the relevance of data collected in geographical locations other than that of the intended release, the applicants' choice of experimental models, the size and statistical validity of experiments – particularly if they deviate from good laboratory practice and internationally accepted standards (OECD, etc.) – the validity of experimental studies conducted using related recipient species, other assumptions embedded in the quality judgements of conflicting studies, and reaching decisions in the presence of identified knowledge gaps (uncertainty).

Again, it is the responsibility of the applicant to produce an ERA that contains sufficient safety-relevant information for regulatory approval in all cases.

### Considerations for the Assessment of Symbiont GMMs

An interesting aspect of the ERA of symbiont GMMs is that, as for their non-GM counterparts, their environmental distribution is determined by the host organism(s) they reside in (in this case arthropods). The extent of the environmental dispersal of a given symbiont will therefore largely depend on its host range. A GM symbiont with a narrow host-range is not expected to colonise other species, thus limiting the possibility of environmental dispersal and unintended effects. The simplest case would be that of an obligate mutualist that lives inside the host cells and is vertically transmitted via the eggs and therefore never resides in external environments. An example of this case could be a GM strain of *Buchnera* sp. inoculated into an aphid (Munson et al. 1991). However, given their difficulty to be genetically modified or even maintained in the laboratory, the use of obligate mutualists for biotechnological applications has been very limited.

When symbionts are horizontally transmitted from host to host they will in many cases reach the external environment. Even if the symbionts depend on the host for multiplication, it does not mean that they cannot survive and persist in a free state. For example, it has been shown that *Wolbachia* sp. is able to survive outside mosquitoes (Gamston and Rasgon 2007), and the same is seen for *Rhodococcus rhodnii*, a reduviid symbiont that enters its host via coprophagy (Durvasula et al. 1997). The symbiont can be exposed to other bacteria both when present within or outside the host, with the possibility of horizontal gene transfer. The symbiont can also be transported and distributed by biotic or abiotic agents. If the microbial symbiont can be propagated *in vitro* on artificial media, it has, at least in theory, the potential to also multiply in natural environments whose conditions are similar to those applied in the laboratory. Hence, and perhaps with the exception of cases such as primary intracellular symbionts like *Buchnera* sp., many GM symbionts may have the potential to be exposed to not only the environments in which it is intentionally delivered, but also to other environments that are within reach of biotic or abiotic agents. Thus, the ERA of GM symbionts may extend well beyond the primary environment in which the GMM is released.

Another significant issue for consideration in the ERA arises when the GMM is confined to a particular host and exerts its function through altered host biology. The genetic modification of the symbiont

may in some cases have little effect of the biology of the microbe itself but result in major changes to the phenotype of the host species. The genetic engineering of insects is in fact sometimes considered as an alternative to the modification of microbial symbionts for pursuing similar objectives (Coutinho-Abreu et al. 2010; Schetelig and Wimmer 2011). For example, the ability to transmit the malarial parasite *Plasmodium berghei* by the mosquito *A. stephensi* can be impaired either via recombinant expression of antiplasmodium enzymes by the gut bacteria *Enterobacter agglomerans* (Riehle et al. 2007) or by genetically transforming the mosquito itself (Moreira et al. 2002). Because the genetic modifications of the microbial symbiont can lead to changes in the host physiology and interactions with other species, the ERA of a GM symbiont may need to include an ERA of the colonised (paratransgenic) host as if it expressed the genetic modification itself. Recent and current efforts in the establishment of appropriate criteria for the ERA of GM insects (Benedict et al. 2010) can therefore assist in the assessment of the safety impact of GMM symbionts.

### Guidance on the Preparation and Risk Assessment of GMM Applications

The EFSA GMO Panel has developed guidance for the risk assessment of GMMs and their derived products intended for food and/or feed use (EFSA 2006). Currently, this guidance document is being updated following experience gained by the Panel while assessing GMM applications for food and feed use in recent years. The purpose of this guidance is to assist applicants in the preparation of applications to market GMMs and their products for food and/or feed use according to Regulation (EC) 1829/2003 (on genetically modified food and feed, EC 2003) as well as notifiers under GMO Directive 2001/18/EC including the ERA and the environmental monitoring plan according to this Directive. In addition, this document provides guidance for the risk assessment of food and feed produced with GMMs, irrespective of whether or not they fall under Regulation (EC) 1829/2003. GMM as pest controllers are not within the scope of the current EFSA GMO Panel guidance. However, several of the described principles for the risk assessment of the genetic modification of the micro-organism and for the ERA of the GMM would apply; namely those relevant for the assessment of: characteristics of the parental organism and inserted sequences, stability of the GMM, toxicity and allergenicity of the introduced trait and environmental

impact of the GMM. In the future, the EFSA GMO Panel might consider development of guidance on GMMs for uses other than food and/or feed in close collaboration with other EFSA Scientific Panels responsible for risk assessment of micro-organisms (e.g. as PPPs).

In addition, the EFSA GMO Panel is currently developing guidance for the ERA of GM insects (<http://www.efsa.europa.eu/en/gmotopics/topic/gmanimals.htm>). Given the above-mentioned overlaps between the risk assessment of symbiont GMMs and GM insects, this guidance will also provide significant input to the establishment of criteria for the ERA of GM symbionts when their phenotype is manifested as changes in the biology of their insect hosts.

### Perspectives

It is expected that applications for release (with commercial purposes or not) of GMM symbionts or arthropods carrying them will be submitted to the EU Member States within the next years. The Federal Drug Administration (FDA) has already authorised three field trials with engineered strains of *Alicagenes* (Bextine et al. 2004) intended to control Pierce's disease in grape grown in the United States ([http://www.epa.gov/biotech\\_rule/pubs/submiss.htm](http://www.epa.gov/biotech_rule/pubs/submiss.htm)). These field trials are, to the authors' knowledge, the only releases of GM symbionts conducted worldwide so far. Another GM endosymbiont that could soon be ready for field trials is the above-mentioned GM *Rhodococcus rhodnii* developed to control Chagas disease in South America (Beard et al. 2001). However, as neither Pierce nor Chagas disease are present in Europe, applications on any of these two products are not expected to be submitted to the EU. With the exceptions outlined above, developments of GM symbiont based products are in their early phases. Therefore, the first application to EU will depend on scientific developments but also on funding opportunities for product development, testing and commercialisation.

It is important that product developers, applicants and European risk assessment and management bodies can rely on adequate legislation and guidance. Advances in developing legislation are already taking place. The biocide Directive is currently under revision to be transformed into a Regulation more in line with the one for PPPs (EC 2009b). In the new biocide Regulation, the interplay with the GMO Directive will be streamlined and the role of ECHA as risk assessment body will be clearly stated. Moreover, the EU GMO legislative framework is being examined by the EC for efficiency and adequacy

([http://ec.europa.eu/food/food/biotechnology/evaluation/index\\_en.htm](http://ec.europa.eu/food/food/biotechnology/evaluation/index_en.htm)). There is nevertheless room for further improvement in legislation. For example, it might be helpful if the GMO Directive would indicate the specific information required for notifications of GMMs. A mechanism providing a single authorisation procedure ('one door – one key') would provide another step forward. Applications for food and feed containing GMOs that are also intended for cultivation fall under the scope of Regulation (EC) No 1829/2003 on GM food and feed and also under the GMO Directive 2001/18/EC. Applicants can choose between submitting separate applications for the two legislations, or submit only a single application under the 'one door – one key' procedure. Under this procedure, the authorisation will cover both human or animal consumption and cultivation. A similar mechanism for GM symbionts intended for release into the environment as plant protection products or pest control systems would streamline the procedure without decreasing the quality of the risk assessment or the level of protection of the European citizens and environment.

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