Bundesgesundheitsbl 2010 · 53:52–57
DOI 10.1007/s00103-009-0986-2
Online publiziert: 27. November 2009

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Environmental risk assessment for medicinal products containing genetically modified organisms

Clinical use of medicinal products can harbour a potential risk for the environment. Active pharmaceutical ingredients, for example, may be excreted by the patient into the environment thereby causing potentially harmful effects on the ecosystem as well as on human health. To evaluate and minimise such risks, a marketing authorisation application (MAA) for a new human pharmaceutical product in the EU requires the assessment of the environmental risks in addition to the evaluation of the product's quality, safety and efficacy. A special regulatory case arises if a human medicinal product contains or consists of genetically modified organisms (GMOs). For marketing authorisation, such a product has to meet the criteria and requirements of both the EU pharmaceutical legislation on the authorisation and supervision of medicinal products as well as the EU environmental legislation on the deliberate release of GMOs.

Legal basis

Any MAA for a medicinal product for human use needs to be accompanied by an evaluation of the potential environmental risks it poses as laid down by Directive 2001/83/EC on the community code relating to medicinal products for human use. This provision is independent on whether or not the medicinal product consists of GMOs. However, the regulatory framework on the environmental risk assessment (ERA) differs between medicinal products that do not contain GMOs and those that do contain GMOs, since the latter additionally has to comply with specific legislation relevant to GMOs. Regulation (EC) No 726/2004 laying down the European Community procedures for the authorisation and supervision of medicinal products for human and veterinary use, specifically requires for GMO-containing medicinal products an ERA similar to the procedure under Directive 2001/18/EC on the deliberate release into the environment of GMOs (**Tab. 1**). For the purpose of this Directive, a GMO is defined as an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination, whereas an organism is defined as a biological entity capable of replication or of transferring genetic material. According to these definitions, recombinant live virus vaccines and many gene therapy medicinal products (GTMPs) such as recombinant oncolytic viruses, replication-incompetent viral vectors, and genetically modified cells or recombinant microorganisms fall within the category of medicinal products consisting of, or containing a GMO (**Tab. 2**).

For medicinal products containing any GMOs, the MAA submitted to the European Medicines Agency (EMEA) has to

Tab. 1 Overview of European legislation and guidance on the ERA of medicinal products and GMOs

Type	Designation	Scope and relevance for ERA
Directive	2001/83/EC	community code relating to medical products for human use → general requirement for evaluation of the potential environmental risk posed by medicinal products
Regulation	(EC) No 726/2004	laying down procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency → requirement for ERA performed in accordance with the principles set out in Annex II and based on information required by Annex III and IV to Directive 2001/18/EC if medicinal product contains a GMO
Directive	2001/18/EC	requirement for deliberate release into the environment of GMOs
Commission Decision	202/623/EC	establishes guidance notes supplementing Annex II to Directive 2001/18/EC
Guideline[a]	EMEA/CHMP/BWP/473191/06 Corr	provides guidance on the ERA for medicinal products containing GMOs
Guideline[b]	EMEA/CHMP/GTWP/125491/06	provides guidance on scientific requirements for the ERA of GTMPs containing GMOs
Guideline[c]	EMEA/CHMP/SWP/4447/00	provides guidance on the ERA for medicinal products for human use that do not contain GMOs

The guidelines can be found at the EMEA website as indicated: [a]http://www.emea.europa.eu/pdfs/human/bwp/47319106en.pdf; [b]http://www.emea.europa.eu/pdfs/human/genetherapy/12549106enfin.pdf; [c]http://www.emea.europa.eu/pdfs/human/swp/444700en.pdf

include an ERA in accordance with the principles set out in Annex II to the deliberate release Directive 2001/18/EC and its supplementing Commission Decision 2002/623/EC. Furthermore, the ERA should be based on the technical and scientific information on the GMO as required by Annexes III and IV to the Directive. The ERA is subsequently assessed as part of the centralised procedure by the CHMP (Co)-Rapporteur. The designated GMO competent authorities of all Member States, in turn, are concomitantly consulted for review of the ERA. This procedure replaces the general requirement of Directive 2001/18/EC for submitting a notification with a technical dossier to the designated GMO competent authorities. Difficulties on preparing the ERA for GMO-containing medicinal products may arise from the fact that Directive 2001/18/EC has a clear focus on the deliberate release of genetically modified plants and agricultural products. Therefore, the EMEA has developed two guidelines, EMEA/CHMP/BWP/473191/2006-Corr and EMEA/CHMP/GTWP/125491/2006, to provide detailed guidance on the preparation of the ERA and to facilitate adaptation of the requirements and the methodology of the Directive to GMO-containing medicinal products (**Tab. 1**).

For authorisation of clinical trials with GMO-containing medicinal products, the ERA needs to be performed according to the national requirements within the Member State in which the trial is performed. The national requirements may vary depending on whether the Member State regards the use of the GMO-containing medicinal product within the clinical trial as contained use according to Directive 98/81/EC amending 90/219/EEC or as deliberate release according to Directive 2001/18/EC. Contained use is defined as any activity with GMOs for which specific containment measures are used to limit their contact with the environment and the general population. Thus, the focus of the contained use Directive lies on the implementation of physical, chemical and biological barriers in accordance with the biosafety level classification of the GMO to preclude its interaction with the environment. In contrast, Directive 2001/18/EC for deliberate release relies

Abstract · Zusammenfassung

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Abstract

Many gene therapy medicinal products and also some vaccines consist of, or contain, genetically modified organisms (GMOs), which require specific consideration in the environmental risk assessment (ERA) before marketing authorisation or clinical trial applications. The ERA is performed in order to identify the potential risks for public health and the environment, which may arise due to the clinical use of these medicinal products. If such environmental risks are identified and considered as not acceptable, the ERA should go on to propose appropriate risk management strategies capable to reduce these risks. This article will provide an overview of the legal basis and requirements for the ERA of GMO-containing medicinal products in the context of marketing authorisation in the EU and clinical trials in Germany. Furthermore, the scientific principles and methodology that generally need to be followed when preparing an ERA for GMOs are discussed.

Keywords

Environmental risk · ERA · GMO · Deliberate release · Medicinal products

Umweltrisikobewertung GVO-haltiger Arzneimittel

Zusammenfassung

Viele Gentherapeutika und Impfstoffe bestehen aus oder enthalten gentechnisch veränderte Organismen, was bei der Bewertung zur Umweltsicherheit dieser Arzneimittel speziell zu berücksichtigen ist. Sowohl vor Marktzulassung wie auch vor Durchführung klinischer Studien wird daher eine Umweltrisikobewertung durchgeführt. Im Vordergrund steht dabei die Frage, ob durch die klinische Anwendung dieser GVO-haltigen Arzneimittel Risiken für die Gesundheit Dritter oder für die Umwelt entstehen. Werden im Rahmen der Umweltrisikobewertung nicht vertretbare Risiken identifiziert, sind Vorkehrungen und Vorsichtsmaßnahmen zu definieren, welche diese Risiken auf ein akzeptables Niveau senken. Dieser Artikel gibt einen Überblick über die Rechtsgrundlagen und Anforderungen bei der Umweltrisikobewertung GVO-haltiger Arzneimittel bei Marktzulassung in der EU sowie bei klinischer Prüfung in Deutschland. Das Konzept und die Vorgehensweise der Risikobewertung werden dabei im Detail vorgestellt.

Schlüsselwörter

Umweltrisikobewertung · Gentechnisch veränderte Organismen (GVO) · Absichtliche Freisetzung · Arzneimittel

Tab. 2 Diversity of GMO-containing medicinal products and risk of shedding

Types of GMO-containing medicinal products	Examples	Risk for shedding of the GMO
genetically modified cells	autologous or allogenic cells expressing a therapeutic transgene	negligible
non-replicating viral vectors	replication incompetent adenoviral, adeno-associated viral, retroviral, or poxviral vectors expressing a therapeutic transgene	low – moderate
genetically modified viruses	recombinant oncolytic viruses like adenovirus, herpes simplex virus, poxvirus, measles virus that may or may not express a therapeutic transgene, recombinant live viral vaccines	high
genetically modified bacteria	recombinant bacteria expressing a therapeutic transgene for oral applications, recombinant live bacterial vaccines	high

on a thorough case-by-case assessment of the potential environmental risks arising from the intentional introduction into the environment of a GMO, and the safety measures necessary to minimise these risks. Some Member States decide on the basis of the specific circumstances of a clinical trial whether a notification for deliberate or contained use is needed. For example, if patients are not hospitalised but treated on an out-patient basis, the deliberate release Directive will be applied. In contrast, if patients remain in a hospital room that fulfils the contained use criteria until release of the GMO has become undetectable, the contained use Directive may be applicable. Another criterion may be whether the administered GMO is released into the environment via the patients' excreta, which is referred to as shedding. In the UK, for example, the contained use Directive is likely to be implemented, if shedding is shown not to occur. However, if significant shedding does, or is expected to occur the deliberate release Directive will be implemented [1].

In Germany, clinical trials with medicinal products containing GMOs are subject to the deliberate release regulations and require an ERA performed in accordance with Annex II and based on information as per Annex III to the Directive 2001/18/EC. The German Medicinal Products Act (AMG) appoints the Paul-Ehrlich-Institut (PEI) for clinical study authorisation of GTMPs and vaccines. If these products contain a GMO, the PEI, in consultation with the German GMO competent authority, the Federal Office of Consumer Protection and Food Safety (BVL), also authorises the deliberate release of the GMO within the clinical trial. In contrast to the administration of the medicinal product itself, any preceding handling of the GMO-containing medicinal products, including its production, transport, storage and preparation for administration, falls within contained use and is, therefore, regulated by the Genetic Engineering Act (GenTG) which implements the EU Directive into national legislation.

Objective of the ERA

The objective of the ERA in accordance with Directive 2001/18/EC is to identify and assess on a case-by-case basis the potential harmful effects of a GMO for humans, animals, plants, microorganisms and the environment at large. Thereby, the ERA should consider any potential adverse effects independent of whether they are direct or indirect and whether they emerge immediately or delayed. If potential harmful effects that pose an unacceptable risk are identified, appropriate measures for reducing this risk need to be defined. Considering the risk reducing measures, the remaining environmental risk is re-evaluated.

GMOs contained in medicinal products may enter the environment at different occasions and by various routes, for example, by unintended dispersal of the product during administration, by accidental dissemination during product handling, by inappropriate disposal of waste or unused product, or via excretion by the patient. Particular attention is given to third parties that are either directly exposed to the product such as medical staff administering the product or are in close contact with the patient such as family members. The patient himself is excluded from the ERA, although effects observed in the patient may be indicative for potential effects of the GMO-containing medicinal product on third parties. If the GMO is transmitted to other persons or the environment at large, the GMO could potentially spread further, undergo genetic or phenotypic changes, compete with existing species or transfer its genetic material to other species. To characterise and evaluate the environmental risks associated with such potential scenarios in its entirety, the ERA should follow the principles and methodology described in Annex II to Directive 2001/18/EC.

Principles and methodology

The ERA in accordance with Directive 2001/18/EC should follow four general principles. First, the GMO should be compared to the non-modified organism from which it is derived. Second, the ERA should be carried out on a scientifically sound premise and rely on known facts supported by data derived from specific testing of the GMO-containing medicinal product including its use in previous clinical trials. If necessary, this data can be substantiated by theoretical assumptions. Third, it is necessary to perform the ERA on a case-by-case basis, since the heterogeneity of the GMO-containing medicinal products and the differences in their clinical use make it difficult to apply standardised requirements or evaluations as part of the assessment. Finally, the ERA needs to be re-evaluated if new information on the GMO or its effects on human health or the environment becomes available.

Information that is indispensable for evaluating the environmental risk include knowledge of the characteristics of the parental and the modified organisms, details on the genetic modifications, effects of inserted or deleted sequences, details on the release and the receiving environment, possible interaction between the GMO and the environment, and information on the monitoring, control, waste treatment and emergency response plans. Experience gained from the release of comparable GMOs into a similar environment can be used to support the ERA.

The ERA procedure should be carried out stepwise as indicated in **Fig. 1**. In the first two steps, the potential adverse effects and their consequences are identified. Step 3 deals with the likelihood of occurrence of the identified adverse effects. This order ensures that initially any potential adverse effect is followed independently of whether it is likely to occur or not.

Step 1: Identification of GMO characteristics which may cause adverse effects

Any hazards or characteristics of the GMO that may result in adverse effects on human health or the environment should be compiled independently of whether they are based on characteristics of the non-modified organism from which it is derived, or arise from the genetic modification. Characteristics that need to be taken into account include the pathogenicity, virulence, infectivity, host range, tissue tropism, replication mechanism, latency/reactivation, survival and stability of the GMO contained in medicinal products. It is useful to start with identifying the hazards associated with the parental/recipient wild-type organism by addressing the pathogenicity, the stability in the foreseen environment, the survival under unfavourable conditions outside the body, for example in the sewage, and the resistance of the wild-type organism to certain disinfectants.

Most GMOs used in medicinal products have been attenuated or modified to reduce the pathogenicity compared to the parental organism. However, attenuated or disabled viruses or bacteria may reverse their attenuating mutations. In this context, it should be kept in mind that attenuated or disabled GMOs in the environment will be under a strong selective pressure for reversion of the restricting modifications. Thus, the origin, nature and stability of attenuating modifications are crucial for the ERA.

Consideration should also be given to the presence of contaminating replication-competent vectors (RCV) within medicinal products consisting of replication-incompetent viral vector suspensions. Unintentional replication competence may arise due to recombination events or trans-complementation in the presence of wild-type or related (pro)viruses. The likelihood of the generation of RCV via homologous recombination events during production may be reduced by avoiding overlapping homologous sequences in packaging cell lines and plasmids used for production. In addition, analytical techniques with appropriate detection limits should be established and incorporated in routine analysis of each batch to detect RCV. This approach is particularly important, if the ERA is based on the replication incompetence of the vector.

Detrimental effects arising from genetic instability of the GMO is another parameter that needs to be addressed in the ERA. The size of the inserted gene(s) may affect the genetic stability of the GMO, as the inserted sequences may enlarge the viral genome to a critical size where it is no longer packaged efficiently. As a result, the genome may become prone to rearrangements. Whereas the loss of the inserted gene(s) is unlikely to constitute a hazard per se, rearrangement of the genome of the GMO could be hazardous. Moreover, the GMO might acquire sequences from another organism thereby increasing its pathogenicity, or, alternatively, it could transfer the inserted gene(s) to another organism. Such unintended transfer of genetic material between different organisms may pose an additional risk factor and should therefore be considered in the ERA.

For identifying the hazards arising from the final GMO, all potential effects of the genetic modifications need to be considered. The inserted gene, for example, might increase the pathogenicity or virulence of the GMO. On the other hand, the deletion of dispensable genes of the wild-type virus could include determinants for immune evasion. While the resulting GMO with lost immune evasion functions could be cleared more efficiently during an infection, acute responses, e.g. inflammation, might be increased. Altered pathogenicity or altered susceptibility to the immune system should also kept in mind if the inserted gene encodes a protein with immune modulatory functions for example, poxviruses expressing interleukin-4 (IL-4), which proved to be more pathogenic in animals than the wild-type virus [2].

Modifications to the viral cellular entry determinants such as pseudotyping, engineering of viral surface glycoproteins, or modifications of host range-determining genes enabling replication of the viral vector in specific cell types are crucial factors that may alter or extend the host range and tropism of the GMO. As a result, the GMO could enter normally refractory cell types, express its inserted gene(s) and, depending on its characteristics, might even replicate within

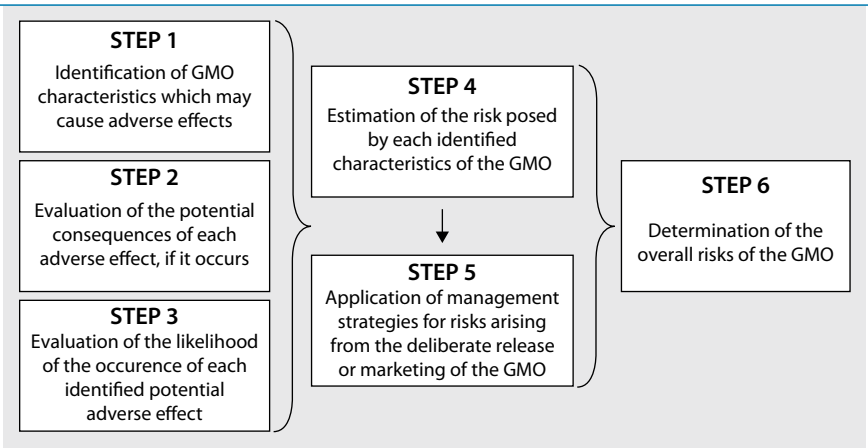

Fig. 1 ▲ Schematic representation of the procedural steps of the ERA for GMO-containing medicinal products. Step 1, 2 and 3 provide the basis for the estimation of the environmental risk in step 4, which takes into account the consequences (step 2) and the likelihood (step 3) of each identified potential adverse effect (step 1). If an unacceptable risk is identified in step 4, appropriate risk management strategies aiming at reducing this risk are applied in step 5. Finally, the overall risk of the GMO-containing medicinal product is re-evaluated in step 6 by considering the estimated risk in step 4 and the applied risk management strategies in step 5

these cells. Such changes may have far-reaching consequences; they could alter the expected route of transmission of the GMO, or lead to unintended expression of the inserted gene(s) at unexpected sites in the patients or in another host. Intervention strategies that can correct these events for example, the availability of effective prophylaxis or an alternative therapy, may markedly improve the environmental safety of the GMO. On the other hand, it has to be ensured that the genetic modification of the organism does not affect its susceptibility to these strategies. The presence of antibiotic resistance genes in genetically modified bacteria or deletion of the thymidine kinase from poxvirus or herpes simplex virus are some obvious examples of how genetic modification might lead to drug resistance of the GMO. The desirable environmental safety properties of genetically modified live oral bacterial vaccines have been discussed in detail in a recent review [3].

Step 2: Evaluation of the potential consequences of each adverse effect, if it occurs

Based on the hazards identified in the previous step, this step defines the magnitude of the consequences of each hazard provided that it does occur. Thereby, the magnitude of the consequences needs to be classified as either high, moderate, low, or negligible for each ecological entity that might be affected. 'Negligible consequences' in this context mean that no significant changes are caused in any of the populations in the environment or in the ecosystems. 'High level consequences' would be any significant changes in one or more species of another organism including endangered and beneficial species that might result in serious negative effects on the functioning of an ecosystem. Consequences for human health are also rated as 'high level' if they cause death, infertility induction, teratogenicity and oncogenicity. In addition to the general public, consideration of the consequences of adverse effects on particular risk groups, such as immunocompromised or elderly persons has to be addressed. The magnitude of the consequences can be further influenced by the conditions and frequency of GMO administration, the exposed environment, and the available measures for limiting an unintended spread of the GMO or reducing or even reversing other adverse effects.

Step 3: Evaluation of the likelihood of the occurrence of each identified potential adverse effect

This step estimates how likely it is that a formerly identified hazard will actually occur. Critical for this estimation are the nature and fitness of the GMO, the characteristics of the receiving environment, the manner and frequency of the release, and the number of released GMOs. For most hazards, however, it may be difficult to make a quantitative estimation of their likelihood. In such cases, the standard high, moderate, low or negligible relative classification system can be used. Another strategy may be to consider a worst case scenario. For such a scenario, all assumptions for lacking quantitative data are maximised to make sure that potential hazards are not underestimated. If the worst case scenario indicates an acceptable risk, then further studies for obtaining quantitative data may be unnecessary.

For GMO-containing medicinal products some specific considerations should be included in this step. First, the ability of the GMO to establish an infection within the patient should be assessed. If an infection can not be excluded, the potential course of the infection including its ability to spread within a community should be taken into account. Thereby, additional information, such as the level of pre-existing immunity in the community or the likelihood for infection of vulnerable groups, should be incorporated in the assessment. Second, the probability that rare events, for example, reversion of an attenuated or disabled GMO to wild-type status will occur should be addressed. Here, the knowledge of the rates of mutation and frequencies of recombination during replication of the GMO are particularly useful. If this information is not available, then experience with similar GMOs may be used. Last but not least, the likelihood of environmental exposure needs to be considered. Procedures that may lead to exposure include the production and preparation of the GMO, administration of the GMO, waste disposal, and shedding. Shedding is difficult to predict [4, 5]; therefore, the release of the GMO after application of the medicinal product should be investigated during the preclinical development in a suitable animal model, as well as in one or more clinical studies. For guidance on such preclinical and clinical shedding studies, a considerations paper on viral shedding has been published by the ICH Gene Therapy Discussion Group. Points to consider for shedding studies include the duration and frequency of monitoring, as well as the type of samples and analytical methods that should be used to determine the extent of viral shedding. Referring back to the parental strain of virus used in the product might provide indications on expected tissue tropism and the likelihood of shedding. The replication competency of the product should also be considered – if the virus can replicate or conditionally replicate, the duration of monitoring should be sufficient to confirm whether or not a secondary peak in viral load and extended shedding periods take place. If the vectors tropism has been altered this might also impact on the route of virus shedding, as might the transgene that has been encoded within the vector. In addition the route of administration and the dose of product to be given might also impact the duration and route of shedding [5].

Shedding of the GMO only represents an environmental risk, if any hazards associated with the GMO have been identified in earlier steps of the ERA. In such a situation, third parties such as family members may be monitored for the presence of the GMO to analyse horizontal transmission of the GMO, which might occur in some situations, as demonstrated for adenoviral vaccine viruses [6].

Step 4: Estimation of the risk posed by each identified characteristic of the GMO

An estimation of the environmental risk of each potential hazard of the GMO should be made by combining the likelihood of occurrence of the adverse effect and the magnitude of its consequences should it occur. This step is complicated due to the quali-

Consequences of adverse effect	Likelihood for adverse effect occuring			
	high	moderate	low	negligible
high	high	high	moderate	negligible
moderate	high	moderate	low	negligible
low	moderate	low	low	negligible
negligible	negligible	negligible	negligible	negligible

Fig. 2 ▲ Risk estimation matrix. Example of how consequence and likelihood for a specific adverse effect may be combined to yield relative estimates of risk

tative terms for likelihood and magnitude of the consequences of an adverse event. However, an indicative risk matrix as illustrated in **Fig. 2** can be used for qualifying the estimated risks. If scientific uncertainty results in uncertainty in the ERA, the potential risk should be maximized in this step. If necessary, adequate safety measurements should be implemented as indicated in the next step.

Step 5: Application of management strategies for risks from the deliberate release or marketing of GMO(s)

If unacceptable risks have been identified in the previous step, risk management strategies should be applied to minimise them. The most fundamental way to eliminate such risks would be to avoid or remove the genetic element leading to the specified risks. However, such a strategy is not always applicable. Instead precautions can be implemented which, in most cases, aim at minimising the likelihood of adverse effects occurring. Simple measures may be to give directions for proper transport, storage, handling and administration of the product. In addition, directions should be given for adequate waste treatment, e.g., inactivation/disinfection of unused product, surfaces, instruments, clothing, gloves, and waste material. Further precautionary measures may include specification for contra-indications such as acute viral infection, sealing of the injection site, collection and decontamination of the patients' excreta, or a requirement for hospitalisation and hygienic measures. While isolation of the treated patient in the hospital is considered an effective measure to avoid dissemination of the GMO, it may not be enforceable since the patient can discharge himself from the hospital. For accidental exposure of the GMO, e.g., spills or needle stick injuries, an emergency plan should be prepared, which defines specific measures outside standard clinical procedures that are appropriate for protection of the environment.

Although the implemented precautions aim to decrease the environmental risk, some uncertainties may persist. Therefore, a monitoring plan should be implemented in addition to the risk management strategies. The monitoring plan should test whether the assumptions of the ERA are correct and whether the risk management strategies are effective. In addition, the monitoring plan should be able to identify unexpected harmful effects that were not anticipated by the ERA.

All necessary directions and warnings concerning environmental safety and safety for medical staff and other contacts during use of the GMO-containing medicinal product need to be included in the product information.

Step 6: Determination of the overall risk of the GMO(s)

Finally, the overall risk of the GMO is evaluated on the basis of the previous steps taking into account the risk management strategies proposed in step 5, which aim at reducing the risks identified in step 4. Thereby, a concise summary of each identified potentially harmful characteristic of the GMO including its overall risk to human health and the environment that may arise from the deliberate release or placing on the market of the GMO should be given. The ERA is closing with the overall uncertainties and a conclusion as to whether the overall environmental impact can be accepted or not.

Conclusion

One of the most central questions for assessing the environmental risks of GMO-containing medicinal products is certainly whether the GMO will disseminate from the patient into the environment or to third parties. Therefore, the inclusion of non-clinical and clinical shedding studies in the development of a GMO-containing medicinal product is particularly crucial for assessing the environmental risk of such a product. If potential risks have been identified in the ERA and if shedding occurs, the risk of horizontal transmission to third parties with close contact to the patient should be additionally addressed. These studies are inevitable to adequately evaluate the overall risk of the product. Finally, only GMO-containing medicinal products, whose overall environmental risk can be considered acceptable, are safe and therefore approvable for clinical trial or marketing authorisation.

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Acknowledgment. This work was supported by a grant of the 7th European Community programme project "PERSIST" to C.J.B.

Conflict of interest. The corresponding author states that there are no conflicts of interest.

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