European Medicines Agency

London, 30 May 2008
Doc. Ref. EMEA/CHMP/GTWP/125491/2006

COMMITTEE FOR THE MEDICINAL PRODUCT FOR HUMAN USE (CHMP)

GUIDELINE ON SCIENTIFIC REQUIREMENTS FOR THE ENVIRONMENTAL RISK ASSESSMENT OF GENE THERAPY MEDICINAL PRODUCTS

DRAFT AGREED BY GENE THERAPY WORKING PARTY (GTWP)	**February 2007**
DRAFT AGREED BY BIOLOGICS WORKING PARTY (BWP)	**January 2007**
DRAFT AGREED BY SAFETY WORKING PARTY (SWP)	**February 2007**
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	**February 2007**
END OF CONSULTATION (DEADLINE FOR COMMENTS)	**August 2007**
AGREED BY GTWP, BWP AND SWP	**April 2008**
ADOPTION BY CHMP	**May 2008**
DATE FOR COMING INTO EFFECT	**November 2008**

KEYWORDS	environmental risk, gmo, gene therapy medicinal product, deliberate release

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 86 13
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

GUIDELINE ON SCIENTIFIC REQUIREMENTS FOR THE ENVIRONMENTAL RISK ASSESSMENT OF GENE THERAPY MEDICINAL PRODUCTS

EXECUTIVE SUMMARY

This guideline deals with the scientific principles and methodology to be used for the environmental risk assessment (ERA) of gene therapy GMO-containing medicinal products for human use, as required for marketing authorisation (MA) under the centralised procedure. Guidance is given on application of the methodology laid down in the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment.

1. INTRODUCTION

Directive 2001/83/EC, as amended, and Regulation 726/2004 require that the applicant evaluates the potential risk of the GMO containing medicinal product to the environment. Therefore an application for marketing authorisation of a medicinal product for human use must be accompanied by an environmental risk assessment (ERA), performed in accordance with the principles of Annex II of Directive 2001/18/EC. Guidance for medicinal products containing or consisting of GMOs appears in the CHMP guideline entitled "Environmental Risk Assessment for medicinal products containing, or consisting of, Genetically Modified Organisms (GMOs)" (EMEA/CHMP/BWP/473191/2006).

2. SCOPE

This guideline focuses on the principles of an ERA for GMO-containing gene therapy medicinal products (GTMPs). Guidance is provided on the scientific principles and methodology to be used for the ERA of a GTMP for human use containing, or consisting of, GMOs, as laid down in Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment. The ERA generally needs to consider potential adverse effects for persons (non-patients) directly exposed to the GTMP, e.g. staff in the clinic involved in administering the product or in patient care; persons who may be in direct contact with the patient, e.g. family members and the 'general public'; and potential adverse effects for animals, plants and micro-organisms, as well as the environment at large. The ERA does not concern any effects on the patient being treated, however effects on patients are important to consider while these might be indicative for possible effects on non-patients that are exposed to the medicinal product.

3. LEGAL BASIS

The legislative framework governing the ERA in applications for marketing authorisation for medicinal products consisting of, or containing GMOs is outlined in previous guidelines (EMEA/CHMP/BWP/135148/2004 and EMEA/CHMP/BWP/473191/2006).

3.1. EU environmental legislation

Directive 2001/18/EC requires that an applicant for placing any GMO on the market as or in a product shall normally submit a Part C notification, including relevant administrative and scientific information, an ERA, a summary, and, if necessary, information on proposed monitoring and risk management strategies, to the designated Competent Authority (CA) of the member state in the territory of which the site intended for placing the GMO(s) on the market for the first time is located. In accordance with a procedure which allows the involvement of the designated CA of each member state and of the European Commission, the notification is examined for compliance with the requirements of the Directive.

3.2. EU pharmaceutical legislation

European pharmaceutical legislation, in the form of Regulation (EC) 726/2004, requires that an applicant for an MA for a biotechnological medicinal product shall submit to the European Medicines Agency (EMEA) a dossier which includes all the necessary administrative, quality, non-clinical and clinical data

necessary for evaluation and approval of the medicinal product. These data are assessed in accordance with the centralised procedure. The active substances of several biotechnological medicinal products are proteins manufactured using recombinant micro- or macro- organisms or cell cultures. However, in most cases the recombinant systems are not themselves components of the finished medicinal product, and as a consequence these medicinal products neither consist of nor contain a GMO.

3.3. The EU environmental/ pharmaceutical legislative interaction for GMOs

Exceptionally, human biotechnological medicinal products may consist of or, more likely, contain, a GMO. These products constitute a special regulatory case by virtue of their registration being governed by reciprocal provisions in the above mentioned Directive 2001/18/EC (Article 12.2) and Regulation (EC) 726/2004 (Articles 6.2 and 6.3).

These provisions require the environmental impact documentation, including the ERA, to be submitted as part of the medicinal product MA application, and to be assessed as part of the medicinal product in a centralised procedure as defined in the Regulation. The ERA is required to be carried out in accordance with the principles set out in Annex II to Directive 2001/18/EC and its supplementing Commission Decision 2002/623/EC, and on the basis of the type of information specified in Annexes III and IV to the Directive. This requirement of the Directive is stated to apply without prejudice to other relevant requirements as regards risk assessment, risk management, labeling, monitoring as appropriate, information to the public, and safeguard clauses provided by community legislation concerning medicinal products for human use (Article 12.2 of the Directive). The general requirement of Directive 2001/18/EC for applicants to submit a notification, including a technical dossier, to the designated CAs is waived in such cases. However, there is a requirement for the CHMP Rapporteur for the application to hold necessary consultations with these bodies and with the European Commission on the GMO-ERA aspects of the procedure.

4. MAIN GUIDELINE TEXT

Underlying principles

An ERA is based on known facts, including those derived from specific testing of the GMO-containing GTMP, as well as on sufficiently underpinned theoretical assumptions, and the precautionary principle, which is described in the Communication from the Commission on the Precautionary Principle. If unacceptable risks are identified during the ERA, risk reducing measures are defined, and a conclusion on the acceptability of the remaining environmental risk is made. This guideline describes the ERA for marketing authorisations. However, the conclusions from a preliminary ERA e.g. for a clinical trial adequate for the development stage of the GMO-containing medicinal product must be taken into account.

In view of the heterogeneity of GTMPs, it is difficult to define general requirements for the ERA that are applicable to all products and therefore the requirements for an ERA should be defined and evaluated on a case by case basis.

An ERA should be carried out based on a scientifically sound premise, empirically derived data and/or clinical use. It should be based as much as possible on quantitative and experimental data obtained with the specific GTMP. Data in the ERA section of a dossier can be derived from, or supplemented by, data from various sources including the quality, preclinical and clinical sections of the dossier. When quantitative data are lacking it may be necessary, and sufficient, to use qualitative data. Where insufficient data are available to assess the actual risk and where experimental assessment in a suitable model has not led to an acceptable assessment, a realistic worst-case scenario based on theoretical assumptions may be used.

New environmental information on the GTMP should be evaluated as data become available. When applying for a MA, the ERA should be up to date and include all relevant information from clinical trials. Relevant new information becoming available after MA should be included in the monitoring plan. Where

new data suggest that an amendment to the terms of the MA is necessary, a variation application should be submitted within the centralised procedure.

4.1. Methodology for evaluating environmental risk

4.1.1. Risk assessment based on experimental data

Unlike ERA for medicinal products that are chemically derived, for GTMP there is no threshold limit about which an environmental risk may be defined. Therefore an environmental risk assessment has to be based on the probability of transmission of the GTMP from the patient to other persons, animals, plants or the environment at large. Experimental or clinical observations may contribute to the ERA.

A GTMP containing a GMO capable of replication and dissemination or transmission could possess an increased risk of being transmitted into the environment**.** Therefore, assays used to demonstrate that the GMO is not capable of replication and dissemination or transmission should have the same or better sensitivity as those used to detect the presence of the GMO in the environmental compartment. Additional experiments may be included to further characterize the GMO found or suspected to be shed. Such experiments will pave the way to decide whether the risks resulting from its transmission into the environment are low or high. They may be used in any of the steps of the ERA described below.

4.1.2. Main principles applying to an ERA

The ERA is based on the following key principles, which are further elaborated on in the sections below:

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

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

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

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

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

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

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

Hazard identification identifies characteristics of the GMO linked to the genetic modification, which may result in adverse effects on human health or the environment. A comparison of the characteristics of the GMO with those of the non-modified organism, under corresponding conditions of the release or use, will assist in identifying any particular potential adverse effects arising from the genetic modification (familiarity principle). It is important not to discount any potential adverse effect on the basis that it is unlikely to occur.

The ERA needs to consider the characteristics of the final GMO containing GTMP, taking into consideration the pathogenicity of the unmodified wild type organism and the nature of the inserted genetic material. Adverse effects may be related to inserted genes and their products, but also to an unforeseen change of the host range or tissue tropism, infectivity, virulence, or latency of the generated GMO. All these effects have to be taken into account, either by making theoretical assumptions based on known science or by experimentally assessing pre-requisites or consequences of such effects.

Identification of hazards associated with the parental/recipient wild type organism

The risks to the environment from a GMO containing GTMP will depend on the nature of the unmodified parental/recipient wild type organism and the effects of any genetic modification. The hazards posed by the application of a GTMP stem from the considerations of the transgene which are put into perspective by the considerations of the recipient organism. The ERA should consider the effect of the genetic modification on relevant characteristics of the recipient organism like for example on infectivity, virulence, host entry/infection mechanisms, cell receptors, replication cycle *in vitro*, replication cycle *in vivo*, occurrence of latency, pathogenicity and mechanisms of transmission. Some of these factors are discussed in more detail in the section on hazards arising from the final GMO.

- Stability:
  Adenoviruses are non-enveloped DNA viruses, which are relatively stable, resistant to dehydration and able to persist in aerosols and water. In contrast herpes simplex virus is an enveloped DNA virus which is highly susceptible to dehydration, and is rapidly inactivated outside of the host. More stable viruses may be resistant to certain disinfectants and may therefore be able to persist in unfavourable environments outside the body, e.g., sewage or non-target hosts. Experimental assessment of the stability of the GMO in the foreseen environment may therefore be required to address this issue.

- Pathogenicity:
  Most organisms used in gene therapy have been modified to reduce pathogenicity of the parental organism, producing either an attenuated virus or bacterium or a replication-incompetent viral vector. The ERA should consider the potential risk of formation of revertants in case of attenuating mutations or the appearance of replication-competent GMOs due to recombination or complementation.

Attenuating modifications

The origin, nature and stability of attenuating modifications are important factors to be considered in the ERA, as they may significantly reduce or alter the pathogenicity of the GMO. In some instances, where the nature of the attenuation may not be well understood, the wild-type or parental organism however may have a history of safe use. For example, many vaccinia virus strains have been modified by passaging, and have not been fully characterised, but have been used extensively in human vaccines.

Sufficient consideration should be given to the possibility that an attenuated or disabled GMO could revert to wild-type status or become competent and able to survive, spread and infect humans.

The loss of an inserted gene from a GMO is unlikely to constitute a hazard per se; however, inherent genetic instability leading to incorporation of genes elsewhere in the genome of the same GMO could be hazardous. Again, it is important to consider that a GMO with a restricted capacity to survive will be under stress in the environment, and there will be a strong selection pressure for the reversion of attenuating and disabling genetic lesions. In case that a GMO will be genetically unstable in the environment outside the patient the ERA should discuss any detrimental effects this might cause.

Replication incompetence

Some viruses that are used as viral vectors, e.g. retroviruses, may raise specific safety concerns, as they might be oncogenic or causing malignant disease, either by insertional mutagenesis of the host chromosomes or as a result of having acquired host oncogenes. These concerns can be reduced if the likelihood of generation of replication competent retroviruses during production of the vector is minimised.

There are available methods, e.g. the use of stably transfected split genome packaging cell lines or self-inactivating (SIN) vectors revealing a minimum of homology to viral genes, which can help to reduce the probability of recombination and insertional mutagenesis events. In contrast, the probability of

homologues DNA recombination may increase when co-transfection methodologies are used that make use of high levels of intracellular plasmid DNA to produce vector stocks.

Cell lines that have been screened for endogenous proviruses will reduce the likelihood of recombination events and mobilisation of endogenous proviruses by super infection with the respective vector. Similarly, the risk of trans-complementation of disabling mutations or deleted genes due to the presence of wild-type or related viruses should be considered. For example, human papillomavirus (HPV) and Epstein-Barr virus proteins have been shown to complement E1A mutant adenoviruses in trans. For larger viruses, such as herpes viruses, there are usually a number of gene deletions, and insertion of the transgene could be into a number of sites. For such vectors, the ERA should include consideration of the transgene insertion site.

Generally it is good practice to demonstrate that vector stocks are devoid of replication competent viruses. Testing for the absence of replication competence should be done as part of routine analysis of each batch. This is particularly mandatory if a risk assessment is based on the replication defective nature of the vector. Direct plating of vector stocks onto permissive cell lines and monitoring of indications of viral replication (e.g. cytopathic effects or syncitia formation) may be used to detect replication competent vector particles; however such approaches do not always give a clear result and specific molecular detection methods such as immunostaining or PCR should therefore be applied.

Identification of hazards arising from the final GMO

A GMO usually contains novel genes compared to the parental/wild type organism. Many modifications will not involve genes with inherently harmful products but adverse effects may nevertheless arise as the result of exacerbation or alteration of existing pathogenic traits. The following potential mechanisms should be considered, although the list is not exhaustive and all modifications should be carefully assessed in the light of known scientific knowledge.

- **Altered pathogenicity**
  An inserted gene could encode pathogenicity or virulence determinant, alternatively the deletion of viral genes that decrease virulence might increase the pathogenicity of the GMO compared to the wild type organism.

  - Inserted preventive, *in vivo* diagnostic or therapeutic genes may encode products with defined biological properties which could be harmful if expressed in a different host, or at levels higher than normal, or under conditions, e.g. in different tissues or at different stages in development, in which they would not normally be expressed. For example, many eukaryotic genes involved in cellular signalling, interaction with the environment, cell cycle control, differentiation or apoptosis may be regarded as potentially oncogenic. Also, residual antibiotic resistance genes may interfere with use of antibiotics in the clinic and should be considered in the ERA.

  - When and how the gene is expressed will affect whether there is any risk to the environment. Stringent control of expression could reduce the environmental risk. Transcriptional control systems need to be described and considered when looking at potential harmful effects of exposure. Expression of potentially harmful genes would not be expected in prokaryotic systems if they were under the control of eukaryotic promoters and vice-versa. Furthermore, inserted sequences can influence the genomic stability of the GMO. When inserted sequences enlarge a viral genome to a size where it is not packaged efficiently, the genome can be prone to rearrangements. This may lead to unexpected changes in behaviour of the GMO and consequently to unexpected effects on the environment.

- **Altered susceptibility to the immune system**
  The ability to evade the immune system is an important determinant of pathogenesis for many micro-organisms. Immune evasion determinants are frequently dispensable for growth in vitro and their deletion can be viewed as innocuous or attenuating. In the event that non-patient persons are exposed to the GMO containing GTMP however, it should be considered that whilst the loss of immune evasion function (for example, deletion of E3 from adenoviruses or the IL-18 binding protein from poxviruses) might result in more effective clearing of the GMO during an infection, acute responses such as inflammation may be increased. Similarly, insertion of genes encoding immune modulatory functions that are not native to the parental organism might affect pathogenesis. For example, poxviruses modified to express interleukin 4 were more pathogenic in an animal experiment than the wild type virus, as this modification led to inhibition of the appropriate immune response for the effective clearance of viral infection. The possible effects (e.g. enhanced proliferation or amplification) of a GMO with impaired immune evasion systems in individuals who may be or become immunosupressed during treatment should also be considered.

- **Altered tropism**
  There are many factors that might change the natural tropism of an organism. Modification or substitution of viral cellular entry determinants can give rise to viruses with altered cellular tropism. Some viruses (for example vaccinia virus) have a number of host range-determining genes that bestow the ability to replicate within certain cell types. Modifications of viral entry determinants (for example viral surface glycoproteins) might permit the entry of the virus into normally refractory cell types and expression of the inserted sequences might occur, even if replication is impossible. Tropism and host range may also be altered through pseudotyping, at least in the vector that is administered to the patient; this will in most cases not apply to the shedded viruses. Pathogenic bacteria may also have determinants that affect host range or the ability to colonise certain sites. The risk assessment should consider possible effects on tissues usually not infected or colonised by the parental organism and whether the normal route of transmission of the parental organism has been altered. The use of replication-competent viruses with an extended/altered tropism would require greater management controls to minimise or prevent a wider environmental exposure.

- **Altered susceptibility to prophylaxis and therapy**
  In the event of exposure to humans, other than the patient, the availability of effective prophylaxis may be an important containment and control measure. Therefore it should be carefully considered, as to whether the modification will result in reduced susceptibility of the GMO to the prophylactic treatment that is effective against the parental organism. For example, this could be additional antibiotic resistance bestowed upon bacteria during the modification process or the conferring of drug resistance to a virus (e.g., deletion of poxvirus or herpes simplex virus thymidine kinase functions leads to resistance to nucleoside analogue-based antivirals). Furthermore, modifications might result in a GMO that has an altered immunogenicity profile. In consequence workers that are immune to the parental organism might then be susceptible to infection with the GMO. Moreover, in such cases, a vaccine that protects against infection by the parent micro-organism might not be effective against the GMO.

- **Unintended transfer**
  The transfer of genetic material of the released GMO containing GTMP to other humans, plants, animals or micro-organisms might be influenced by i) the conditions, e.g. scale and dose, of the release, ii) the availability of susceptible hosts for the gene therapy product, iii) ability of the GMO to replicate and iv) the potential for recombination of the genetic material within the GMO.

  Transfer of gene sequences is not a harmful process by itself. During the hazard identification process, it is important to consider the potentially harmful consequences of sequences inserted

into a GMO being transferred to other organisms, or that the GMO itself may acquire sequences that might increase its pathogenicity. There are many mechanisms by which sequences may be transferred between organisms. Factors that affect the frequency of gene transfer leading to a harmful consequence are complex and require careful consideration in the risk assessment.

Step 2. Evaluation of the potential consequences of each adverse effect

The magnitude of the consequences of each potential adverse effect should be evaluated. This evaluation should focus on the hazards that have been identified in Step 1 of the ERA. This evaluation should start from the supposition that such an adverse effect will occur. The magnitude of the consequences is likely to be influenced by the environment into which the GMO(s) is (are) intended to be released and the manner of the release.

The severity of the consequences of each identified adverse effect needs to be qualified, e.g., in terms of magnitude ranging from high, moderate, low to negligible. These consequences are influenced by the genetic constitution of the GMO, the exposed environment, the health status of those likely to be exposed, the method of administration and the frequency of use of the GMO. Some considerations are:

- The spread of the GMO containing GTMP in the environment or its host species. This might be affected through i) a changed biological fitness (e.g. increased competitiveness), ii) ways of dispersal of viable material (e.g. transport, way of administration, shedding), iii) environmental, e.g., climatological conditions (temperature sensitivity).

- Interactions with other organisms. Examples include the exposure of personnel that are involved in the study and in the treatment of the patient, or the interaction with other microorganisms that are present in the patient.

- Effects on population dynamics or genetic diversity in the receiving environment, e.g., if a GMO has an increased growth rate compared to wild-type organisms.

The ERA needs to consider direct and indirect, immediate and delayed effects of the final GMO. An example of a direct effect could be accidental exposure to a GMO which is modified to deliver a human growth factor or a bacterial toxin: expression of these genes in non-target tissues could have potentially harmful consequences. However, direct effects may also be delayed in onset, making them more difficult to be linked to the GMO containing GTMP. For example, an immune response against a GMO in a subject other than the patient is a direct consequence of shedding. However, such seroconversion may take time to be detectable. Oncogenesis could theoretically be a direct effect of exposure to certain types of GMO but might be hard to link to the GMO if the onset of clinical symptoms occurs at a later date.
An example of an indirect effect is a GMO which compromises prophylactic, therapeutic or diagnostic procedures, through dissemination of antibiotic resistance. Another example could be a new infectious agent arising from recombination or complementation events after release of the GMO.

Step 3. Evaluation of the likelihood

The likelihood of the occurrence of each identified potential adverse effect is evaluated in Step 3 of the ERA. A major factor in evaluating the likelihood or probability of adverse effects occurring is the characteristics of the environment into which the GMO containing GTMP is intended to be released, and the manner of the release.

The risk assessment process thus far has involved identifying those features of the GMO containing GTMP that have the potential to cause harm and the mechanisms by which these hazards could be realised. Next, the likelihood that the identified hazards will be manifested should be evaluated. Theoretical scenarios can be drawn up which suggest that a GMO containing GTMP may be hazardous to people, but the chances of these scenarios being realised are often small. The ERA should focus on realistic scenarios.

The study of realistic scenarios may however involve the use of a worst case scenario. Worst case scenarios are very useful to identify and evaluate possible harmful effects that arise from the use of a GMO containing GTMP, when a quantitative scenario cannot be made, due to lack of quantitative data. In a worst case scenario the assumptions for which no quantitative data are available, for example assumptions on exposure, are maximized. A worst case scenario is useful as it yields a maximum appraisal of the potential hazards, without the need for supplying detailed empirical information, which may be difficult, and very costly. The use of detailed information may not make the risk assessment essentially more valid than the worst case approach. If the worst case approach does not lead to a conclusion that the risk is unacceptable, then a 'less than worst case' would also not lead to such a conclusion.
If, for instance, the worst case approach for application of a vector may lead to the following conclusion: 'shedding may occur and may lead to infection of people in the neighbourhood of the patient: this would not lead to any effect, because the vector is attenuated (replication defective) and expression of the inserted gene will not have a negative effect; the risk of this scenario is negligible.' The use of this worst case scenario precludes the need for extensive experiments to gain more empirical data needed to assess the actual level of shedding that may occur.

In the evaluation of the likelihood of the occurrence of a potential adverse effect, factors that come into play are (i) judgment on the overall fitness of the GMO and (ii) the probability that rare events may occur (e.g. the likelihood of gene transfer). Issues relating to the likelihood of harm arising will be very difficult to handle in situations where there is no firm data on which to make a judgment; however, many wild-type viruses have co-existed for thousands of years without jumping species. Nevertheless, caution must be applied when seeking to discount any potentially harmful properties of the GMO containing GTMP on the basis that they are unlikely to be manifested. In general, the credence given to information used in these considerations should reflect the quality of the supporting data. In other words, where there are insufficient data on the GMO containing GTMP at hand, it would be prudent to perform experiments. If the data on which an ERA should be based are still incomplete, theoretically assume the worst case scenario in the ERA and act accordingly.

Consideration of the ability of a GMO to establish an infection *in vivo*
An assessment should be made regarding the ability of the GMO to establish an infection, how efficient that infection would be and its ability to spread within the patient, and therefore within a community. This represents an evaluation of the 'fitness' of a GMO and should be based upon established scientific knowledge rather than assumptions, where possible. If the gene therapy vector is attenuated, it may not be infectious even if it can enter target cells.

It is important that fitness is not coupled with pathogenicity per se as some modifications whilst theoretically making the GMO more pathogenic may also render the GMO less fit. For example, consider the insertion of a foreign gene into the E3 locus of adenovirus. The modified virus will be less likely to establish an infection and spread in the community as the loss of E3 makes the virus more susceptible to immune surveillance and therefore reduces the risk of environmental spread.

For many gene therapy vectors it should be acknowledged that there is widespread immunity in the human population, which is likely to reduce the ability of the GMO to spread into the community. For example, in Europe, most people will have immunity to adenovirus serotype 5, although young children may be more susceptible. The level of immunity in the community may be an important consideration in the ERA, but the data need to be carefully considered, particularly in relation to vulnerable groups.

Consideration of the probability that rare events will occur
It may be possible to assign a frequency to a given event. Often, this can take the form of a precise numerical frequency obtained in-house or through published data. For example, the rates of mutation and frequencies of recombination during viral replication are open to quantitative analysis and are known and published for many viruses.

In many cases, however, this will not be possible and an approximate, semi-quantitative or descriptive assessment of the frequency, based upon experience with similar GMOs or techniques can be used. For example, the likelihood of an attenuated or disabled GMO reverting to wild type status can be assessed on the basis of the number of discrete events that would need to take place, i.e. the more events needed, the less likely it is that reversion will occur.

It should not be assumed, however, that failure to observe or detect an event is evidence that it does not occur, i.e. these results should be presented as 'chance is less than', not as 'chance is 0'. As part of such considerations it should be recognised that micro-organisms often have extremely short generation times and therefore adapt to specific environments and selective pressures rapidly. This is particularly true for viruses and during the course of evolution they have proved particularly adept at responding to selective pressures by infecting new cell types or host organisms.
For each potential adverse effect, conditions of administration to the patient (dose, route of administration) and conditions of potential exposure of the environment (magnitude, duration) should be evaluated.

The route of administration of the GMO containing GTMP will influence environmental exposure. In general the exposure will be higher if for instance, the GMO is administered via a nasal spray than if the GMO is administered orally or by injection. The site and mode of injection (e.g. intramuscular, intravenous) will influence systemic distribution, and therefore shedding.

The magnitude of the exposure depends on characteristics of the surrounding environment, including medical staff members, social contacts and the general public. Procedures that might lead to exposure, include i) production and preparation of the GMO; ii) administration and iii) waste disposal. Shedding is an important factor in the exposure of the environment to the GMO. It is recommended to incorporate shedding studies, i.e. analyses if the GMO is released after GTMP application, in an animal model into the non-clinical medicinal product development program, and also in one or more clinical trials in the clinical development program. Generally the purpose of clinical trials are to study the adsorption, distribution, metabolism and excretion of one or more investigational medicinal products with the object of ascertaining its (their) safety and/or efficacy (directive 2001/20/EC; definition of a clinical trial). As such the evaluation of vector shedding is a requirement for a phase I study. Pre-clinical biodistribution and shedding data can be used to define which tissue samples are to be taken and the timing of sampling pre- and post-administration. For example urine, faeces or mucosal nasal swabs, could be analysed as a part of a biodistribution study for the presence of the GMO. The assays used should be state-of-the art and highly sensitive. It is acknowledged that determination of shedding might be hampered by limitations conferred by the instability of many GTMPs in unfavourable matrices such as urine. Stability issues will therefore have a major impact on the reliability of the obtained results. Additionally it is advised to take care of the respective samples and provide the necessary measures to ensure stability and GMO traceability.

If the presence of a GMO has been shown, e.g. by detecting its genome via polymerase chain reaction (PCR), shedding and the potential for transmission into the environment may be assumed. PCR negativity may indicate the absence of the GMO in particular environmental compartments. It should be noted that PCR will only detect the presence of GMO genomes without giving any information about the infectivity. Ideally, if positive DNA signals are observed the samples should be followed up for infectious virus quantification. Combinations between culturing and PCR detection may yield an improved rate of detection. The data derived from shedding studies should be used in the environmental risk assessment. It is noted that an ICH Considerations paper on shedding is under development and will provide additional guidance, when available.

Shedding by itself is not considered an adverse effect. Increased shedding resulting in greater environmental exposure only leads to a risk if there are significant consequences that have been identified earlier in the ERA. An example could be replication-incompetent viral vectors carrying a transgene used to treat prostate cancer. Shedding/excretion of the product in urine could be significant, whereas the environmental risk could be negligible as the virus is unable to replicate. In the case of a replicating virus

this may be quite different. Furthermore, lytic viruses such as herpes and vaccinia may cause surface lesions, leading to viral shedding. This may be managed by using attenuated non-lytic derivatives, or through management of the lesion, such as the use of appropriate dressings. Such issues need to be considered in the ERA, and an appropriate strategy adopted.

Step 4. Estimation of the risk

An estimation of the risk to human health or the environment posed by each identified characteristic of the GMO which has the potential to cause adverse effects should be made as far as possible, given the state of the art, by combining the likelihood of the adverse effect occurring and the magnitude of the consequences, if it occurs.

To estimate the environmental risk, the magnitude of the consequences of the identified potential adverse effects is combined with the outcome of the evaluation of the likelihood. The risk may also be described in qualitative terms ranging from high, moderate, and low to negligible. While it is not possible to multiply qualitative terms, a risk matrix is useful as a tool that illustrates the process of risk estimation.

In the estimation of risk the level of scientific uncertainty of the risk assessment should be taken into account. Scientific uncertainty may have been taken into consideration sufficiently, for instance by the use of worst case scenarios, as explained above. However, if scientific uncertainty still exist and leads to uncertainty in the risk assessment, it should be taken into consideration in this step, by maximizing the potential risk that may occur in the risk scenario, and taking adequate safety measurements accordingly. This procedure is an application of the precautionary principle (*reference to the opinion of the European Commission).*

Step 5. Application of management strategies

The risk assessment may identify risks that require application of a risk management strategy.

In a clinical trial situation, risk management (e.g. containment of patients) is potentially more easily achievable, compared to the situation when MA has been granted and the use of the medicinal product is no longer fully controlled by the applicant. Therefore, in the interest of a smooth transition from the clinical trial stage to a marketing authorisation, it may be prudent to start collecting data on the feasibility or lack of necessity of implementing risk management strategies at an early stage.

Precautions can be implemented to decrease an environmental risk. Risk management strategies may affect i) the characterised hazard, ii) the consequences of the hazard occurring or iii) the estimated likelihood. In most cases the likelihood is reduced. Some examples of risk management measures are:

i) Specification of contra-indications, e.g. acute viral infections or a compromised immune status;

ii) Requirement for hospitalisation and hygienic measures. Isolation effectively reduces environmental risks but creates a problem if a patient decides to leave the hospital early. Furthermore, the patient population of a hospital may be more than normally vulnerable to the GMO making it important to prevent infection and environmental spread in the hospital.

iii) In cases of parenteral administration leakage can be reduced by sealing the injection site.

iv)Control measures to minimise aerosol formation: adenoviruses are robust and transmitted effectively in aerosols and droplets, even if disabled or attenuated. Aerosol producing operations should therefore be reduced during preparation and administration procedures.

The effectiveness of risk management strategies can be evaluated by identifying the step in the ERA that is affected by the risk management. Precautionary measures should decrease the estimated risk but be proportional to the seriousness of the risk. Some remaining uncertainty is inevitable. Medicinal products

are subject to post-authorisation monitoring and such pharmacovigilance may contribute to environmental risk management. Two types of monitoring must be included in the design of a monitoring plan.

> i) Case specific monitoring which should verify the hypothesis described in the environmental risk assessment and monitor the risk management measures that have been introduced to reduce the identified risks for the environment.
> ii) Monitoring plans should be suited to observe unexpected effects with a possible impact on environmental safety of the medicinal product which have not been included in the environmental risk assessment.

It is strongly recommended to incorporate the monitoring plan in existing monitoring methods e.g. pharmacovigilance. New data emerging from this monitoring should be evaluated for their impact on environmental safety. Management measures to reduce the risk to an acceptable level should be described in the Summary of Product Characteristics (SPC).

Emergency plan. Several aspects of the use of a GMO containing GTMP may require an emergency plan, e.g. needle stick accidents. The ERA should indicate if special measures, outside standard clinical procedures (e.g. sharps bins) are required to keep environmental risks at an acceptable level. Where necessary, guidance should be provided in the SPC for dealing with environmental safety and safety for medical staff and other contacts during use of the GMO containing GTMP.

Waste treatment. It may be necessary to use disinfectants and subsequent waste treatment. Environmental issues that result from the use of the disinfectant itself are not part of this ERA. It should be noted that disinfectants themselves are also subject to licensing for use in individual member states so it may be necessary to determine if the disinfectant of choice is actually permitted in the concerned member state. The following should also be considered: disinfection of materials used for transport, preparation or administration; including surfaces, instruments, hoods, clothing, and gloves, efficacy of the disinfection methods or agents proposed, measures taken following a spill. Furthermore, clinical waste containing GMOs should be inactivated before disposal using suitable methods.

Post-authorisation monitoring. The following should also be considered: expected effects on other humans; on animals and/or plants; on the environment; conditions for release of treated patients; monitoring plan or reasons for not installing a monitoring plan; monitoring of methods installed to prevent spread to medical personnel or people in the environment.

Step 6. Determination of the overall risk

An evaluation of the overall risk of the GMO(s) should be made taking into account any risk management strategies which are proposed.

Based on the previous steps a conclusion should be given as to whether the overall environmental impact is acceptable or not. This final evaluation should be expressed as a summary of the overall risks that are connected to the specific gene therapy application.

DEFINITIONS

Environmental risk. In the framework of medicinal products, mainly the risk of transmission of the GMO to humans other than the patient, to animals or to the environment at large. A secondary risk may be posed by pathogens, which arise by recombination with the original GMO.

Monitoring plan. Following deliberate release into the environment, directive 2001/18 requires that "general surveillance" and "monitoring as appropriate" are performed. A monitoring plan should include systemic distribution and shedding (if shedding data must be included also depends on the outcome of the environmental risk assessment), surveillance of long term side effects, information about adverse or

unexpected effects, monitoring effects in others than the patient and information about off label use. An overview of sampling strategy and analytical methods to be used should be given.

REFERENCES

• Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms.

• Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use.

• Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency.

• Communication from the Commission on the precautionary principle, COM (2000) 1 final, dated 2.02.2000. http://ec.europa.eu/environment/docum/20001_en.htm

• Council Decision of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

• Council Decision of 3 October 2002 establishing pursuant to Directive 2001/18/EC of the European Parliament and of the Council the summary information format relating to the placing on the market of genetically modified organisms as or in products.

ABBREVIATIONS

GMO	Genetically Modified Organism
GTMP	Gene Therapy Medicinal Product
SPC	Summary of Product Characteristics
MA	Market authorisation
ERA	Environmental Risk Assessment
CA	Competent Authority
GM	Genetically Modified