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# Genetically Engineered Virus-Vectored Vaccines – Environmental Risk Assessment and Management Challenges

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## 1. Introduction

Genetically engineered or modified viruses (GMVs) are being increasingly used as live vaccine vectors and their applications may have environmental implications that must be taken into account in risk assessment and management processes. In most legislative frameworks GMVs are treated as GMOs (genetically modified organisms), which require ERA (environmental risk assessment) in addition to the evaluation of the quality, safety and efficacy of the product before marketing authorization or clinical trial applications are submitted. The ERA is performed in order to identify the potential risks for public health and the environment that may arise due to the use and release of GMVs. If risks are identified and considered as not acceptable, the ERA process should go on to propose appropriate risk management strategies capable to reduce these risks (Anliker et al., 2010; Kühler et al, 2009).

To obtain marketing authorization within the EU, a GMV has to meet the criteria and requirements of the EU pharmaceutical legislation for both medical and veterinary applications, as well as the EU environmental legislation on the deliberate release of GMOs. Hence, although viruses are not organisms, it will be necessary to perform an ERA similar to the procedure under Directive 2001/18/EC on the deliberate release of GMOs into the environment. For the purpose of an ERA, an organism is defined as a biological entity capable of replication or of transferring genetic material, and this definition will then include viruses and also replication-incompetent viral vectors. A MAA (marketing authorization application) for a GMV submitted to the European Medicines Agency (EMEA) has to include an ERA in accordance with the principles set out in Annex II Directive 2001/18/EC and its supplementing Commission Decision 2002/623/EC. The further process is well described in recent reviews, e.g. by Anliker et al. (2010) and Kühler et al (2009). Briefly, the ERA should be based on the technical and scientific information about the GMV as required in the Directive Annexes III and IV. The continued procedure is somewhat different from the deliberate GMO release process of the Directive. But even so, difficulties in preparing ERAs for GMVs may arise from the fact that Directive 2001/18/EC has a nearly exclusive focus on GM plants and agricultural products. Therefore, the EMEA has developed two specific guidelines for the preparation of ERAs to facilitate adaptation of

the requirements and the methodology of the Directive to GMO-containing medicinal products. In addition, the GMV ERA needs to be performed according to the national Member State requirements to obtain authorization of GMV clinical and of field trials. The individual Member State may have different requirements, e.g. dependent on whether the trial is considered "contained use" according to Directive 98/81/EC or a "deliberate release" according to Directive 2001/18/EC. The former is focusing on containment measures, i.e. implementation of physical, chemical and biological barriers to preclude the GMV-environment interactions. In contrast, the deliberate release Directive is based on a thorough case-by-case assessment of the potential environmental risks arising from GMV release or escape, and the biosafety measures to be utilized in order to eliminate or minimize these risks.

The objective of an 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 (domestic and wildlife), plants, microorganisms and the environment at large (Anliker et al., 2010). Potential adverse effects should be acknowledged and considered irrespective of whether they are direct or indirect and whether the emerging effects appear immediately or delayed. When such effects have been identified, appropriate measures for their reduction or elimination need to be defined. Such measures have to be based on the realization that transmission of GMVs to non-target individuals, species or the environment at large may allow the GMV to spread further. In its turn this may induce genetic or phenotypic changes, competition with existing species or horizontal gene transfer of hereditary materials between species. To fully conceive and evaluate the environmental risks associated with such, and other, potential scenarios, detailed case-by-case knowledge of all possible adverse effects of a given GMV is crucial, because the quality and relevance of the ERA, and the possibilities for efficient risk management, are totally dependent on the ability to anticipate, predict and reveal potential adverse effects. This again is dependent on the society's willingness to invest in "what-if?"-based and precautionary science and in acknowledging and weighing the values that are knowingly or unknowingly influencing the decision making process following a MAA for a GMV application.

The main focus of risk-related research has previously been on the functionality and the intended immunological mechanisms of GMVs, while work on safety aspects, particularly in relation to ecosystem effects, often have been put off until later in vaccine development. By then, making fundamental changes to the vaccine in order to improve its safety can be extremely costly and time-consuming. In some cases hazards and irreversible harms may have been initiated already. Hence, we will argue that risk assessment and management should not be considered as two separate processes.

Traditionally, risk assessment has been considered as a "scientific" process, while risk management and communication has included value judgments with regard to acceptability, the trade-off criteria and the adaptation of strategies for coping with uncertainty. However, risk assessments are influenced by scientific, ethical, economic, social and political information. For instance, risk assessments include value judgments both with regard to consequences that should be avoided and the process of risk characterization. Consequently, risk assessment and management strategies need to be connected from the very start of a vaccine development project in order to unveil the full spectrum of environmental impacts.

In this article we describe GMV applications and the environmental impact questions and challenges that are connected to them. We then proceed to discuss the relevance and shortcomings of the present risk assessment framework and how this framework needs to

be better connected to risk management strategies. Such management strategies are developed within particular frameworks that need to include awareness to normative standards and preferences regarding human relation to the natural environment. Moreover, we will elaborate on how precautionary motivated research involves the need to advance hypotheses about GMV specific harm and hazard endpoints and that such endpoints are dependent on both the objectives of ERA and of the management strategies.

## 2. Creation and applications of GMVs

Genetically engineered or modified viruses (GMVs), from a number of taxons, are being increasingly used as live vaccine vectors. The so far approved veterinary vaccines have most commonly been based on replication-competent canarypoxvirus or herpesviruses, and EMEA has published a guideline for Live Recombinant Vector Vaccines for Veterinary Use.

There are 4 broad GMV application areas that may have environmental implications:

i. Immunization against infectious diseases in livestock species;
ii. Immunization of wild life species which are reservoirs of infectious agents causing disease in humans and livestock species;
iii. Control of pest animal population densities by either direct lethal control operations or immuno-contraception; and
iv. Human vaccination programs against infections diseases or cancers.

In all cases there may be circumstances that enable GMVs to jump species barriers directly, or following recombination with naturally occurring viruses. All the different applications may, to varying extents, represent release or unintended escape of GMVs into the highly varying ecosystems.

The different application areas call for different considerations and options with regard to choice of virus vectors and genetically engineering (GE) strategies. Generally spoken, there are two strategies: The first is represented by *gene-deleted viruses* to be used for homologous vaccination, i.e. to achieve protective immunity against the GMV itself. The engineered deletions most commonly target genes that are necessary for the virus to carry out a full multiplication cycle, or are implied in viral virulence. Furthermore, "non-essential" genes may be deleted in order to obtain markers for monitoring unintended vaccine virus spread. Lack of the deleted marker gene will indicate that a field virus isolate originate from a GMV. A number of gene-deletion GMVs for vaccination against human and livestock diseases have been marketed, or are in the final stages of clinical trials. Most of them belong to the herpesvirus or adenovirus families.

Recombinant virus vectors obtained by transgenesis represent the second strategy. Such viruses are created in cell cultures by simultaneous transfection with a plasmid carrying a gene from the virus/microbe that is to be immunologically targeted, and infection with the virus vector of choice. The plasmid construct is such that the transgene contains DNA sequences homologous to a viral gene in each end. Hence the transgene is transferred and integrated to a predetermined site in the virus vector genome by homologous recombination. The most commonly used vector viruses are members of the DNA virus families *Poxviridae* and *Adenoviridae*. In many cases the vectors have been engineered by both endogenous gene deletions and transgene insertions.

At present there are considerable research efforts going into designing *replication-incompetent* versions of the most attractive vector viruses. This is possible under the qualification that the non-replicating vector is able to express the vaccine transgene and raise protective immunological responses at the same levels as the parental replication-competent virus strain. The main purposes are to minimize the risk-prone possibilities of productive infections with shedding of virus in the excreta of GMV-exposed individuals, and reversal of attenuated or modified viruses to full virulence through reversion of attenuating mutations.

Diseases for which GMV-vectored vaccines have been developed or are in the process of being developed, include AIDS, severe acute respiratory syndrome (SARS), Epstein-Barr virus, cytomegalovirus, West Nile virus, tuberculosis, malaria, influenza (human and equine), Rinderpest, Rift Valley fever, borreliosis, trypanosomiasis, leishmaniasis, cervical cancer, breast cancer, colorectal cancer, mesothelioma and melanoma. Most of the GMV vectors employed are assumed replication-incompetent, and are derived from members of the poxvirus and adenovirus families.

## 3. Vaccines and vaccination (modified from Traavik, 1999)

All vaccines have in common the intention to prevent disease or limit the effects of disease. Both humoral (antibody-mediated) and cellular arms of the immune system can contribute to a pathogen-specific acquired response that distinguishes specific immune protection from the innate and more general protection mediated by phagocytes (i.e. macrophages neutrophils and dendritic cells), cytokines and physical barriers. Because vaccination against a threatening disease may take place many years before exposure to the pathogen, immunological memory is a critical element. A long-lived immune response, which may be mobilized and augmented rapidly when called for, is essential.

Vaccination may have different purposes and fields of application. The most important are:

- Protection against and treatment of infectious diseases
- Protection against and treatment of cancer
- Induced infertility in domestic animals and wildlife

An ideal vaccine provides an optimal mobilization of the adaptive immune system with no unwanted side effects, and with long-lasting immunological memory. The most universal purpose is to prevent disease in individuals and prohibit transmission of disease agents between individuals. Generally, vaccination must be carried out before the individual becomes infected, but for some diseases, e.g. rabies, disease may be prevented even if vaccination takes place after infection.

Some important human and domestic animal pathogens, e.g. rabies virus, hantaviruses and a number of arboviruses, have reservoirs in free-ranging wildlife animals. Human and animal disease may then be prevented by vaccination of reservoir animals. Likewise, some free-ranging mammalian species are considered «pests» in the context of human food, animal fodder or other kinds of production. Enforced infertility following vaccination is now becoming an alternative to culling ("stamping out") for control and reduction of such pest animal populations.

Cancer cells often express surface antigens not present on their normal counterparts. Such unique antigens may provide targets for vaccines that may induce immune reactions to prevent and combat cancer cells.

Depending on the species, target-organs, epidemiological considerations etc., the vaccine delivery method and route may differ. In practical terms, the vaccine may be delivered by:

- Injection, most commonly intramuscularly or subcutaneously. The recent development of injection, so-called "gene guns" are used to propel small gold particles covered with antigen through the skin. Such procedures are often referred to as "biolistics".
- Inhalation of vaccine-containing aerosols.
- Ingestion of vaccine-containing vehicles, i.e. capsules.
- For fish: Bathing in or spraying with vaccine containing solutions
- For free-ranging animals: Baits containing vaccines that are spread out over the selected target area from airplanes or helicopters.

To a varying extent, all vaccine delivery strategies imply that vaccine-containing materials may end up in unintended locations, and hence release or escape of biologically active macromolecules (i.e. DNA or RNA), viruses or microorganisms into the environment may take place.

## 4. Viruses that are used as GMVs

Generally spoken, the families most widely employed as GM vaccine vectors have been members of the *Poxviridae*, *Adenoviridae* and *Herpesviridae* families. The frontrunners have been GM versions of the poxvirus genera Orthopoxvirus and Avipoxvirus. Our own experimental and sustainability oriented research have focused on these genera, and we will in section 5, 6 and 7 proceed by using such GM vectors as examples of ERA and risk management challenges.

There is now a tendency that GM viral vaccines are based on vectors that are replication-incompetent in the target species of choice. Such vectors may be selected on the basis of natural host species barriers, e.g. avipoxviruses with birds as natural hosts, and assumed replication-deficient in mammalian species. Other commonly used vectors have been made replication-incompetent by cell cultivation procedures (e.g. MVA, see below) or targeted genetic engineering, e.g. adenovirus vectors.

Veterinary vaccines against infectious diseases containing replication-competent GMVs have been on the market in the EU since the beginning of year 2000 (Kühler et al., 2009). Several vaccines for human use are currently in various stages of clinical trials, and a few of them, mostly based on adenovirus or poxvirus vectors, have already been approved for marketing.

### 4.1 Poxviruses as GM vaccine vectors

Our account will henceforth be directly relevant for poxvirus vectors, but at least in a general sense it will also be relevant for GMV vectors developed from other virus families.

Poxviruses are selected as potential vectors for several reasons (Liu, 2010): the extensive use of the smallpox vaccine (and the related modified vaccinia ankara), which provided knowledge of the human safety parameters; the large gene capacity for the insertion of a heterologous gene encoding the antigen of interest; the broad tropism of the virus for mammalian cells that could then result in a number of cells expressing the vaccine protein (heterologous antigen); the production of vaccine protein for a relatively short period of

time (making the kinetics of the production of the heterologous protein more akin to antigen production from an acutely infecting pathogen, but less useful for gene therapy applications); and the location of the virus in the cytoplasm, thus avoiding integration risks that might occur with other alternatives, as a retroviral vector for example (Mastrangelo et al., 2000). The earliest vector for delivery of a heterologous antigen is a licensed veterinary vaccine employing a MVA (Modified Vaccinia Ankara) vector to deliver a rabies antigen (Mackowiak et al., 1999). The vaccine was developed for delivery as bait for wild animals. The success of using a vector to deliver an antigen that generated immunity sufficient to protect animals and curtail outbreaks in the wild, via oral delivery of bait (hence with imprecise dosing), in a variety of animal species, demonstrates many of the advantages of an ideal vector, in this case efficacy, safety, and even ease of distribution (oral delivery), which would not have been as facile with a non-vectored vaccine. Oral delivery may mobilize protective local immune responses on mucous membranes, a great asset for human, domestic animal and wildlife vaccines.

Various poxvirus vectors have undergone human clinical testing as HIV vaccines. In the largest clinical trial, a canarypox vector coding for HIV antigens was utilized as the first component of a prime-boost regimen in a clinical trial of an HIV vaccine involving 16,000 individuals (Rerks-Ngarm et al., 2009). In another prime-boost vaccination protocol for prophylaxis of HIV, the priming is being done with a DNA plasmid, followed by an MVA vector boost

(http://clinicaltrials.gov/ct2/show/NCT00820846?term=DNA+%2B+MVA+for+HIV&rank=5).

### 4.2 Vaccine history, general characteristics and taxonomy of the family Poxviridae

The bicentennial celebration of the first vaccination took place 15 years ago. In 1796 Edward Jenner injected cowpox virus into the boy James Phipps, and later on challenged him with fully virulent human smallpox (variola) virus. The boy survived, and Jenner had hence protected him against one of the most dreaded human diseases of all times. The smallpox vaccination story ended in a triumphant eradication, in 1979, of variola virus and smallpox disease, due to a world-wide vaccination campaign. (Fenner et al., 1988). In recognition of Edward Jenner´s contribution, procedures that aim at protection against disease by pre-mobilization of the immune system were termed "Vaccination", derived from the Latin word *vacca,* hence honoring the cowpoxvirus. In that context it is well worth for present day scientists to reflect on the fact that the contemporaries of Jenner rejected his findings.

The family *Poxviridae* contains a vast number of complex DNA viruses that replicate in the cytoplasm of vertebrate and invertebrate hosts. The member viruses have been sorted into two subfamilies based on their host preferences, namely, the *Chordopoxvirinae* (infecting vertebrates) and *Entomopoxvirinae* (infecting invertebrates, more specifically arthropods). The *Chordopoxvirinae* consists of eight genera with different host ranges, namely, Orthopoxvirus, Parapoxvirus, Avipoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus and Yatapoxvirus. The *Entomopoxvirinae* is divided into three genera based on the insect host of isolation. These are designated A, B, and C entomopoxviruses, respectively. Genetic information on the entomopoxviruses is very scanty.

The distinctive characteristics of members of the family *Poxviridae* include:

- A large and complex virion (larger than any other animal virus particles) with virion-associated transcriptase used for virus-specific mRNA synthesis. The virions are very

resistant to environmental degradation. This is an advantage for the shelf life of vaccines, particularly in areas with unsatisfactory cooling conditions. But persistence in the environment may also enhance the chances of transboundary movements and non-target infections.

- A large genome composed of a single linear double-stranded DNA molecule, 130-300 kbp, with a hairpin loop at each end. The genome facilitates insertion of one or more transgenes with a collective size of up to 20-30 kbp. Vaccine vectors carrying a number of vaccine transgenes may hence be constructed. Theoretically, a single vaccine may protect about a number of diseases that are prevalent in specific geographical areas or defined age groups.
- The complete viral multiplication cycle takes place in the cytoplasm of the host cell, without presence in the latter's nucleus. This is of course a major biosafety asset of poxviruses, since it precludes the insertion of viral DNA into the host genome, and the putative adverse effects that may arise by such events.

The poxviruses are diverse in their structure, host range and host specificities. Members of each of the 8 genera of the subfamily *Chordopoxvirinae* are genetically and antigenically related and share similarities in morphology and host range within the genus. For example, similarities in the restriction endonuclease maps of several Orthopoxvirus genomes, such as the 90% sequence identity of the genes of Vaccinia and Variola viruses reflect genetic relatedness of members of each genus. It is, therefore, not surprising that intra-genus cross-hybridization has been reported among these viruses.

Genetic divergence exists between members of each genus, particularly when such members come from geographically separated econiches. Indeed, Weli et al. (2004) even observed genetic heterogeneity among avipoxviruses isolated from different parts of Norway.

Sequence similarities of genomic repeats in various strains of each virus species suggests that these repeats evolved from unequal crossover events. Thus, genetic material of each virus bears a pointer to genetic recombination in its history. Further examination of the Orthopoxviruses shows evidence of genetic transpositions and deletions in the terminal hypervariable regions of the genome. The significance of these genetic interactions or phenomena in the ecology of poxviruses is at present a matter of conjecture but they makes risk assessment in the use and release of poxvirus-based GMVs absolutely necessary.

### 4.3 Ecological distribution of *Poxviridae*

The family *Poxviridae* contains a vast number of divergent viruses with equally divergent host specificities and host ranges. It seems justified to state that poxviruses will be found in any vertebrate species and geographical area where they are systematically looked for. However, very little is known about the characteristics and geographical distribution of most poxviruses occurring naturally in the field. This knowledge would be necessary if a meaningful risk assessment of poxvirus-based GM vaccines use or release in any target area should be undertaken. Without such knowledge there is no possibility of making meaningful ERA inclusions of adverse effects due to recombination events between GM poxvirus vaccines and naturally occurring poxviruses circulating within the actual area for application.

Many poxviruses are capable of zoonotically infecting man. It is likely that variola virus is derived from an ancient zoonotic virus that originated from a now extinct animal host

species. In general, poxviruses show species specificities that range from narrow to broad, but *we still know little about the fundamental mechanisms that mediate the host tropism of individual poxviruses* (McFadden, 2005). The unpredictability in a real world situation is illustrated by a macaque outbreak caused by a European orthopoxvirus strain carried by *Rattus norvegicus* in the Netherlands (Martina et al., 2006).

Variola virus (smallpox disease) has killed more members of the human population over the span of recorded history than all other infectious diseases combined. Variola virus may never again infect humans, but there are other poxviruses that can cause serious human disease. In 2003, an outbreak of human monkeypox occurred in the mid-western United States due to the inadvertent importation of monkeypox virus in a shipment of rodents from West Africa (Reed et al., 2004). Fortunately, the strain that caused this outbreak was more benign in humans than the more pathogenic variant that is found in central Africa, which results in mortality rates of 10–15%.

Variola virus, prior to its eradication, spread all over the world. Similar worldwide distribution seems to be true of VACV and Molluscum contagiosum virus. Man was the only known host and reservoir of Variola and Molluscum contagiosum viruses. Although the reservoir host of VACV is unknown, it has been shown, under natural conditions, to exhibit a wide host range among humans, rabbits, cows and buffaloes. Other poxviruses with worldwide distribution include numerous species of Avipoxvirus. Even with the relatively limited studies so far done, certain orthopoxviruses (parapoxviruses, capripoxviruses and yatapoxviruses) are notably associated with African domestic animals and wildlife, including rodents, squirrels and monkeys, which may serve as reservoirs of infection. Knowledge of the distribution of poxviruses that infect man and with host range which includes wildlife such as rodents, squirrels and monkeys, would be important in risk assessment of poxvirus-based GE vaccines and other poxvirus-based recombinant constructs prepared for use or release in ecosystems to which these hosts belong. Similarly, it is important to document the poxviruses carried by ectoparasites of animals in the target localities.

### 4.4 Poxvirus vaccine vectors

Poxviruses are being increasingly used as recombinant vectors for vaccination against numerous infectious diseases in humans, domestic animals, and wildlife (Pastoret & Vanderplasschen, 2003). For risk assessments and surveillance, information about the occurrence, distribution and ecology of poxviruses are hence important.

Poxviruses have several advantages for use as expression or vaccine vectors, including their large insertion capacity, their cytoplasmatic site of replication, their heat stability, the relatively high expression levels and proper post-translational modifications of foreign proteins as well as the ability to raise protective immune reactions locally on mucous membranes.

Vaccinia virus (VACV) strains are robust eukaryotic expression vectors that have been used for a number of different studies in biochemistry, cell biology, and immunology. Most vaccinia virus strains, such as the ones used in the smallpox eradication campaign, readily replicate in human cells and have been associated with a range of clinical complications in vaccinees. This fact is presently considered a major problem for the use of these strains as recombinant vaccines for mass vaccination. For laboratory use, in addition to mandatory biosafety level 2 procedures, proper precautions must be taken to prevent accidental exposure when managing replication-competent vaccinia strains.

### 4.4.1 Non-replicating poxvirus vectors

To circumvent the problems associated with "classical" vaccinia virus strains, several laboratories have been increasingly involved in efforts to develop more attenuated, host-restricted virus strains (for brief review, see McFadden, 2005). Generally, these efforts involve two related strategies: i). The isolation of chicken cell-culture adapted vaccinia virus variants that are replication-deficient in mammalian cells, for example MVA, or ii). The development of avipoxvirus platforms, such as canarypox (ALVAC) and fowlpox (TROVAC), supposed to be naturally non-permissive for mammalian cells. There is increasing evidence that such non-replicating vaccines are safer than the original vaccinia strains and are still comparably immunogenic.

#### 4.4.1.1 MVA (Modified vaccinia virus Ankara)

Among the so-called "non-replicating poxvirus vectors", modified vaccinia Ankara (MVA) has elicited considerable interest because of its excellent safety record in humans, its ability to mobilize different protective arms of the immune system, and the possibility to raise local mucosal membrane immunity after oral delivery. In addition to being a promising vector for the construction of poxvirus-based recombinant vaccines, one appealing feature of MVA as an expression vector is that it can be used under biosafety level 1 laboratory conditions. MVA has in common with NYVAC and other adapted VACV strains that they are *supposedly not carrying out fully productive infections in "relevant" mammalian cell types.* Such VACV strains do, however, secure efficient expression and presentation to the immune system of protein products from transgenes inserted into different locations in the viral DNA genome.

MVA was derived from a Turkish smallpox vaccine strain (Ankara) (reviewed by Drexler et al., 2004). After more than 500 passages in chicken cells, it tested defective for replication in human cells and avirulent in lab animals. From 1968–1980, MVA was inoculated into more than 100,000 individuals in Germany with no reported secondary complications and it is now considered to be a suitable platform for the next generation of safer smallpox vaccines and recombinant poxvirus vectors. Genomic mapping and sequencing studies have revealed that MVA lost nearly 30 kb of genomic information during its extended passage in chicken cells. Furthermore, it has multiple deletions and mutations compared with the parental strain. Many of these genetic alterations were in host-response genes. It is assumed that these deletions render MVA unable to carry out productive infections in human cells.

MVA has been proven very efficient in induction of mucosal immunity. This is of course utterly important since a number of human and animal disease agents use mucosal surfaces as their portal of entrance to the organism.

#### 4.4.1.2 Avipoxvirus vectors

Like MVA, canarypox virus (ALVAC) and fowlpox (TROVAC) vaccine vectors induce antibody and cytotoxic T-cell responses, critical in the immune defense against viruses, to vectored viral antigens in a range of mammalian species (Hel et al., 2002; Srinivasan et al., 2006). Replication of avipox virus vectors is regarded abortive in mammalian cells, eliminating the safety concerns that exist for replication competent vaccinia virus vectors. The avipox viruses infect mammalian cells and produce viral proteins, with the replication block occurring at the time of viral DNA synthesis. It should, however, be noted that the experimental data backing claims for total lack of avipoxvirus replication in mammalian

cells are based on studies embracing very few viral species or strains, and also very few, and mostly virus-ecologically irrelevant, mammalian cell cultures. For some avipoxvirus/mammalian cell combinations fully productive infections have been demonstrated (Weli et al., 2004).

Licensed ALVAC-vectored vaccines for dogs, cats, horses, chicken and ferrets are commercially available, and an ALVAC-vectored HIV-1 vaccine has entered phase III clinical trials. Some additional ALVAC-vectored HIV-1 and some human cancer and malaria vaccines are in clinical phase I or II trials (Weli & Tryland, 2011).

### 4.5 Current research on risk assessment of GMVs

There is little peer-reviewed information available that relates to ERA of GMV releases. To our knowledge research related to environmental effects is only being performed for alphaherpesviruses (Thiry et al., 2006) and poxviruses (orthopox and avipoxviruses). Such environmental biosafety-related research has been performed for a number of years in Norway, but we have no present knowledge of other research groups with a similar focus. We have concentrated on biosafety issues of the orthopoxvirus strain MVA (Modified Vaccinia Ankara), considered to be a very safe vaccine vector because of high gene expression capacity and lack of viral replication in mammalian cells (Drexler et al., 2004).

The most relevant conclusions from our studies may be summed up as follows:

- Orthopoxviruses, and hence potential recombination partners for orthopoxvirus vectored vaccines, are common in different small rodent species populations all over the country, and small rodent predator species are infected by and have antibodies to such viruses (Sandvik et al., 1998, Tryland et al., 1998).
- Recombination between an influenza-transgenic MVA and a naturally occurring orthopoxvirus is readily demonstrated in cell cultures. The recombinants may have phenotypic characteristics, some of which may point towards adverse effects, different from the parental viruses. Recombinants may be genetically unstable and "throw out" the influenza transgene. This will eliminate the most logical tag for vaccine monitoring, and will also diminish the ability of the vector to mobilize protective immune responses (Hansen et al., 2004).
- The absolute and relative permissivities for MVA multiplication and viral shedding have not been thoroughly studied. GM and unmodified MVA may, contrary to the general dogma, perform fully productive infections in highly relevant mammalian cell types, and other mammalian cell cultures that are semi-permissive to infection (Okeke et al., 2006).
- DNA sequencing revealed that orthopoxviruses can be clearly separated into geographically distinct strains, and it was inferred that these strains have distinct evolutionary histories in different rodent lineages (Hansen et al., 2009). It is an open question whether these different virus strains have aberrant abilities to engage in recombination events with GM vaccine vectors.
- Upon sequencing of an orthopoxvirus isolated from a human clinical case, it was established that this strain was a naturally occurring hybrid between two distinct orthopoxvirus species. This is the first proof of concept for orthopoxvirus recombinations taking place under authentic ecological circumstances (Hansen et al., 2011).

- Homologous recombination between orthopoxvirus-vectored vaccine and naturally circulating orthopoxviruses, genetic instability of the transgene, accumulation of non-transgene expressing vectors or hybrid virus progenies, as well as cell line/type specific selection against the transgene are potential complications that may result if poxvirus vectored vaccines are extensively used in animals and man (Okeke et al., 2009a).
- Phenotypic characteristics of recombinants between GM and naturally occurring orthopoxviruses may be unpredictably different from any of the parental viruses (Okeke et al., 2009b).
- Contrary to common assumptions, some avipoxviruses may carry out productive infections in mammalian cells, and avipoxviruses within a restricted geographical area may be more genetically diverse than realized so far (Weli et al., 2004 and 2005).

## 5. Features by GMVs that are relevant for risk assessment and management frameworks

Directive 2001/18 with annexes, the EMEA guidelines and CPB with annexes give a number of leads towards GMO characteristics that may indicate hazards or adverse effects. Characteristics that must be taken into consideration (Anliker et al., 2010) include the pathogenicity, virulence, infectivity, host range, tissue tropism, replication strategy, latency/reactivation, survival and stability of the GMO. Here we will point to two important features by risk assessment and management frameworks that may cause difficulties when preparing ERA and risk management strategies for GMVs.

### 5.1 The distinction between chemicals and organisms

Risk perspectives used on chemical pollutions have been influential when shaping risk concepts in biosafety related to genetically modified organisms (GMOs), and hence also GMVs. We will argue that, and discuss why, this starting point is of very doubtful relevance to self-replicating organisms and molecules.

When the first genetically modified plants were approved and released the adequacy of the present framework for assessment of chemical substances i.e. ecotoxicological assessment, for GMOs was contested (Meyer, 2011). Chemicals have a release-dependent concentration decline with a given breakdown time in the environment, while GMOs follow a different environmental routes and degradation pathways than chemical pollutants. (Trans)Genes follow the path of the host genome, possibly eventually also the path of sexually compatible and some incompatible species (through vertical and horizontal gene transfer). Thus for GMOs exposure do not necessarily predict response, and accordingly risk models (based on the premise that exposure dose predicts response) have no or little utility in predicting the environmental behaviour of released transgenes. This was illustrated for example when Hilbeck et al. and Losey et al. reported adverse effects of Bt toxins and Bt maize pollen on non-target organisms in laboratory experiments (Hilbeck et al., 1998, Losey et al., 1999). To improve ecotoxicological testing, Andow and Hilbeck (2004) have suggested that investigation of non-target effects of GMOs may be done more efficiently by employment of a combination of two models; Ecotocixology testing of chemicals and risk assessment of non-indigenous species. However, the ecotoxicological approach still remains the recommended one in risk assessment procedures of GMOs although the new EU biosafety Directive 2001/18/EC partly supports the ecological approach since it prescribes a more detailed ERA.

### 5.2 The distinction between viruses and organisms (modified from Traavik, 1999)

It is important to keep up the distinction between viruses and organisms. Viruses are *not* organisms. Furthermore, the differences in genome strategies and life cycles between virus families are often more fundamental than between different mammalian or plant families.

Viruses multiply intracellularly in permissive host cells. One single virus particle (virion) infecting a permissive cell may give rise to billions of new particles during a short time (hours to days). The submicroscopic size of virions and the ability to spread over vast, even global, distances during short timespans are important, basic conceptions for ERA and risk management of any GMV. This is important in order to ask the relevant harm and hazard related questions, and hence to realize and conceive the risks connected with a given GM vaccine virus in a specific ecosystem and society context.

In addition to such fully productive infections, some virus/host cell combinations may result in persistent infections with virus shedding in the excreta for extended periods, while others lead to latent infection with viral DNA in a host chromosome-integrated or episomal state. Latent infections may be intermittently reactivated and accompanied by virus shedding. Integration of viral DNA into the host cell genome may by itself have harmful consequences, irrespective of viral gene expression or replication. The same virus strain may, under differently modulating conditions, display all these life cycle forms.

The host tropism, at the species-, organ- or cell type-level, is quite narrow for some viruses, while others have a much wider host-spectrum. For most viruses the molecular, genetic and epigenetic pathways determining host-cell specificity are not known in detail. Restrictions may be present at various steps during a virus multiplication cycle, from the lack of cell membrane receptors to subtle incompatibilities with host cell enzymes necessary for viral nucleic acid transcription and replication. Hence, minor genetic changes taking place during or after engineering of GM viruses may have profound effects on the host tropism and ability to spread to non-target host species.

For many virus/host cell combinations permissivity is a relative term, since it may be influenced to a considerable extent by the menu of genes expressed by the cell, and by the exact levels of gene expression. In culture, the permissivity of a given host cell may be manipulated experimentally by activation of intracellular signal transmission pathways, i.e. by hormones, growth factors, cytokines etc. Such procedures may also enhance persistent or reactivate latent infections. At the intra- as well as at the inter-species level of host animals this is illustrated by a vast variation in susceptibility for a given virus strain. Such variation may be related to sex, age, mating season, pregnancy, genetic differences, infection with other viruses or microorganisms, and environmental factors promoted by season, climatic changes or by pollution, e.g. EDCs (endocrine disrupting chemicals).

It is important to be aware of the distinction between viral infection and viral disease. An infected individual may shed virus and represent a transmission reservoir without showing clinical symptoms. Yet, other individuals within the same or other species may become clinically ill, or the viral infection may result in abortions, stillbirths, teratogenic or oncogenic effects. For persistent/latent infections, clinical symptoms may be present intermittently, only under special circumstances, or appear a long time after infection.

Different strains of the same viral species may have different virulence or pathogenicity, as well as host-cell or -species tropism. Even genetic differences at the single point mutation level may result in virus strains with aberrant phenotypic characteristics.

For GMVs it is hence conceivable that unintended phenotypic characteristics with unwanted ecological consequences may be established in addition to the intended modification(s). This may not become evident unless very comprehensive and carefully planned experiments and ecosystem surveillance/sampling programs are carried out. In many instances fully adequate studies are totally precluded by the complexity and the regular or occasional variations of the recipient ecosystem. Hence scientific uncertainty or ignorance is a state that must be accepted.

## 6. The connection between risk assessment and risk management

According to Anliker et al. (2010), an ERA based on 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."

We find it an interesting and elucidating statement that "data can be substantiated by theoretical assumptions". This opens the ERA gate for creation and inclusion, as well as theoretical, mathematic and experimental modeling, of hypotheses build on "Worst case scenarios", as pointed out by Kühler et al. (2009), and also for the use of stringent Precautionary principle versions in situations and processes dominated by scientific uncertainty and ignorance (see section 9).

The case-by-case and step-by-step approaches to GMV ERAs seem to be instituted in all relevant national and international legislations, including EU Directive 2001/18/EC and the Cartagena protocol on biosafety (CPB). Any ERA should include comparative data about characteristics of the GMV and its unmodified parental virus strain. Details about the genetic modification/engineering process, including data about effects, genomic location and DNA sequence of the transgenic (vaccine gene) insert must be present. Descriptions of the vaccination regime and release/possibilities for escape of the GMV must be considered. The receiving environment/ecosystem, including possible interactions between the GMV and the environment, have to be described. Plans for monitoring, control, waste treatment and emergency response plans have to be prepared and presented, and this is, of course, tasks that overlap with risk management activities.

Anliker et al. (2010) state: "Experience gained from the release of comparable GMOs into a similar environment can be used to support the ERA". In our view this is to some extent an unwanted premise for trustworthy ERAs since it opens up for subjective interpretations of "comparable GMOs" and "similar environment". Theoretical considerations and practical experience indicate that such parameters rarely exist for engineered organisms, and are even more rare for viruses. This should become evident while reading and reflecting on the scientific ignorance, uncertainty and lack of knowledge related to the nature, characteristics and ecology of GMVs.

The ERA procedure may be scientifically and operationally divided into five steps, whereby the ERA and risk management is integrated and coordinated (described in an excellent review by Anliker et al., 2010):

1. Identification of potential adverse effects;
2. Evaluation of the potential consequences of each adverse effect, if it occurs;
3. Evaluation of the probability (likelihood) that each identified potential adverse effect should occur;
4. Estimation of the risk posed by each identified characteristic of the GMO;
5. Application of risk management strategies related to marketing or deliberate release of the GMO;
6. Evaluation of the overall risks of the GMO, based on the conclusions from the previous steps, taking into account the risk management strategies proposed in step 5, which were created to reduce or eliminate the risks identified in step 4.

Through the outlined procedure each potentially harmful characteristic of a GMO should be turned into risks. The total process should form a basis for consideration of the overall risk to the ecosystem, animal and human health by deliberate release or marketing of a given GMO. The ERA should conclude on whether there is a need for a risk management plan. If necessary and possible the ERA should devise appropriate risk mitigation methods. And of primary importance: The ERA should reach a conclusion as to whether the overall environmental impact is at all acceptable or not (Kühler et al., 2009).

Step 1 of the ERA procedure is focusing on the identification of GMO characteristics that may result in adverse effects. This is the decisive step for whether, and to which extent, the ERA and the risk management plans will provide biosafety and make contributions to sustainable development as well as to good health of ecosystems, animals and humans.

In our opinion the creative conception and design of "What-if?"- and Worst-case-scenario-inspired ideas, questions and working hypotheses, must be encouraged and stimulated in order to enhance the chances of high precaution levels in ERA and risk management processes. Furthermore, we will argue that these goals will only be met if independent scientists and institutions carry out the relevant intellectual processes and research projects. Finally, although national legislations, and also the Cartagena protocol, require documented scientific evidence as a basis for ERAs and risk management plans, implementation of the Precautionary principle is always expressively required (Kûhler et al., 2009). The different versions, interpretations and possibilities for implementation of the precautionary principle are further discussed in section 9.

### 6.1 Relevant risk assessment and management questions for GMVs

The different virus families have their specific life cycles and host-preferences. Hence it is impossible to make risk assessment schemes that are valid for all potential virus vectors. Risk assessment must be performed on a case-by-case, step-by-step basis, taking into account the characteristics of the ecosystem into which the virus will be released, and the ability of the virus to engage in transboundary movements (Traavik, 1999; McFadden, 2005).

The most evident risk issues related to release of GMVs or unmodified viruses are the known and unknown unknowns related to (i) whether active multiplication with virus shedding in excreta takes place in target individuals, (ii) infection of non-target species, (iii)

recombinations with naturally occurring virus relatives and (iii) integration of GMV DNA into host cell chromosomes.

Ideally, before running the risk that any GMV becomes implanted into a species/population/new location/ecosystem a number of crucial questions should be answered (modified from Traavik, 1999), e.g.:

- Can the released virus engage in genetic recombination, or by other means acquire new genetic material? If so, will the hybrid offspring have changed their host preferences and virulence characteristics?
- Can the released virus or any hybrid or mutated offspring be shed in the excreta of intentionally or unintentionally infected individuals?
- Can the released virus or any hybrid or mutated offspring infect unintended (non-target) species?
- Can the released virus or any hybrid or mutated offspring integrate into the genomes of host cells?
- Can other viruses that are present within the ecosystem influence the infection with the released virus or its offspring?
- Can insects or migrating birds or animals function as vectors for the released virus or its offspring, to disseminate viruses out of their intended release areas?
- May climatic changes and/or xenobiotic pollutants, e.g. EDCs (endocrine disrupting chemicals), influence the virus/host animal/ecosystem interactions?
- For how long can the virus and its offspring survive outside host organisms under realistic environmental and climatic conditions?
- Are the virus and its offspring genetically stable over time?
- Can the virus or its hybrid or mutated offspring establish long-lasting, clinically mute, persistent or latent infections in naturally accessible host organisms?
- Can the virus or its offspring activate or aggravate other naturally occurring latent or persistent virus infections?

Some of these questions deal with the genetic and phenotypic characteristics of a supposedly genetically stable GMV. But the situation becomes even more complex and unpredictable if the GMV parental strain under certain conditions or circumstances is genetically unstable, giving rise to viral strains with altered characteristics (Traavik, 1999). At the present time it is strongly needed that questions and hypotheses related to the influence of climatic changes and xenobiotics, e.g. endocrine disrupting chemicals (EDCs) are included in ERAs and risk management plans.

Demanding ERA and risk management challenges connected to all the questions raised in this section are related to whether methods allowing detection of the phenomena listed, and still unlisted, have been developed, and whether the surveillance and monitoring programs that make their employment possible have been funded and operationalized.

## 7. Biosafety Implications for environmental risk assessment and management frameworks

We will argue that in order to harvest the potential benefits of any GMV, the approaches used in the Norwegian orthopoxvirus studies should be part of the regulatory risk governance frameworks all over the world (see section 4.5 as well as 6.1). In relation to ERA

and risk management needs, some of the most urgent issues for targeted research will be treated in the following.

### 7.1 Naturally occurring relatives

With GMV use and release, it will be crucial to determine the occurrence, distribution and ecology of poxvirus relatives. In Norway rodents and other small mammals are considered to be natural reservoirs for different orthopoxviruses, and cowpoxvirus-like strains in particular (Sandvik et al., 1998). Approximately 20% of shrews and small rodents belonging to eight species carry orthopoxvirus DNA sequences in their organs, and 20% of such animals have specific serum antibodies as a sign of previous infections.

### 7.2 Potential recombination events and their consequences

If a GM orthopoxvirus infects an individual, animal or human, that already carries another orthopoxvirus, a recombination event, homologous or illegitimate, may follow. Foreign genes may hence be transferred from the GM donor virus to other GM poxvirus vaccines or to wild type orthopoxvirus recipient strains (Sandvik et al., 1998). The outcome may be hybrid viral progenies with unpredictable pathogenicity and altered host range. The probability and possible outcome of recombination is dependent on the characteristics of the viral vector used, and the occurrence of naturally occurring or genetically modified relatives.

In cell culture co-infection experiments with a MVA-based human influenza vaccine and a newly isolated Norwegian cowpox virus-like strain, a number of recombinant progeny virus strains were obtained (Hansen et al., 2004). One of the progeny strains displayed phenotypic characteristics different from both parents, and was genetically unstable upon cell culture passage, i.e. the influenza transgene was deleted at a high frequency. This is of paramount monitoring significance, since the inserted gene will always be the marker of choice for tracing and detection of vaccine-related effects.

Environmental release or escape of a GMV provides the opportunity for recombination with poxviruses present in target and non-target animals, including humans. In most parts of the world the occurrence and distribution of naturally occurring poxviruses are virtually unknown. But, in central-Africa it has been proposed that the vaccinia- rinderpest vaccine (RVFH) might recombine with a pox-relative, namely monkeypoxvirus. Since smallpox has been eradicated, the monkey pox is at present the most feared poxvirus (Reed, 2004), and has for instance caused virulent outbreaks in Zaire (Kaaden et al., 2002).

Under natural circumstances the probability of recombination may be low, but predictions and forecasts are made impossible by the fact that we lack knowledge about the natural occurrence and prevalence of poxviruses in all parts of the world. Furthermore, even when recombination events are rare, the consequences may be serious since one viral progeny particle may multiply into millions of identical particles in a matter of hours. In this context, it is disturbing that the present regulatory frameworks are based on a one time risk assessment of any given GMV, and does not take into account the putative consequences that may arise from successive releases of the same, or related, GMVs.

During the global smallpox eradication program (in the 1970s) transmission from vaccinated to unvaccinated persons was estimated to occur at a rate of 27 infected per million total vaccinated (Centre for Disease Control, 1991). It has been possible to isolate the vaccinia

virus from domesticated animals that have been in contact with recently vaccinated humans. The potential host spectrum of vaccinia virus is very broad and includes laboratory animals, pigs, cattle, camel, and monkey species (Fenner, 1996). In the past, vaccinia virus has for instance spread from vaccinated humans to domestic animals such as cattle (through milkers) and buffaloes, before spreading within the herd. Furthermore, it has been reported that during outbreaks of buffalopox, the buffalopox virus infected unvaccinated humans (Dumbell & Richardson, 1993). In Brazil, an emerging virus with similar characteristics as a smallpox vaccine virus caused disease both in cattle and their human caretakers (Damaso et al., 2000).

GMVs made for wildlife vaccination must be thermostable and have long environmental persistence, and VACs satisfy these criteria. The vaccinia-rabies vaccine (VRG), for instance, has been reported to persist 4 months under natural conditions without a significant loss of viral infectivity (Brochier et al., 1990). Environmental stability is a prerequisite for herd immunity in target animals, but by the same token it increases the risk of non-target effects, and for spread to non-target species and other ecosystems.

VACV has been shown, in cell cultures, to easily engage in genetic recombination with other orthopoxvirus species (Ball, 1987). A high number of closely related viral species are known, and high degrees of sequence homology across species borders have been demonstrated. It may, however, be safely assumed that a high number of unknown orthopoxviruses are circulating in many different types of ecosystems and biotopes all over the world. Biological (i.e. insects, migratory birds and animals, domestic and pet animal trade, infected individuals in an incubation period etc), as well as mechanical, (i.e. automobiles, airplanes) vectors may enable further dissemination and transmission of a virus to other ecosystems. Changed tropism for particular cells or tissues may result in divergent virulence in the target, or other known, host species for the GMV or its recombinant progenies compared to the parental, unmodified poxvirus (Traavik, 1999). In addition, the GMV or its recombinant progenies may have had their host restriction programs changed, so that the viruses can transfer to and infect formerly resistant host species. Other phenotypic changes may also happen. For instance, it has been reported that GE of a poxvirus created a GMV that was more virulent than the non-modified virus. As part of a strategy to develop pest animal contraceptive vaccines, the gene encoding human interleukin-4 (IL-4) was inserted into the Ectromelia virus (mousepox) genome (Jackson et al., 2001). Expression of IL-4 was intended to curb unwanted anti-viral effects. Unexpectedly, genetically mouse pox-resistant mice infected with the IL-4 expressing virus developed symptoms of acute mouse pox accompanied by high mortality.

### 7.3 Non-target effects and transboundary spread

GMV administration may have non-target effects. Baits containing GMVs may be eaten both by target and non-target animals. In the second instance, viral spread may also take place by direct/indirect human/animal contact, or by installation of the GMV into a food web. A non-target GMV transfer was illustrated by the account of a woman, with a predisposing skin disease, who, having removed a VRG bait from the mouth of her dog, became infected and developed pox lesions (Rupprecht et al., 2001).

The eradication of rabies by vaccination may have indirect consequences for the ecological balance; lack of disease favours an increase in the host animal population, in this case the European red fox. Therefore, it is crucial that monitoring of GMVs in the environment is

initiated with the purpose to follow-up the performed risk assessment, to map the actual environmental effects and to identify unforeseen adverse ecological effects.

GMVs or their progenies resulting from mutations and/or recombination events may achieve new phenotypic traits of importance for their ecology, spread and host preferences. The opportunities for long distance spread and cross-species transfer of mammalian viruses have increased in recent years due to enhanced contact between humans and animal reservoirs. It is, however, difficult to predict when such events will take place since the viral adaptations that are needed are multifactorial and stochastic. Recent examples of viruses that have crossed species barriers are HIV, hantaviruses, haemorrhagic fever viruses, various arboviruses (e.g. West Nile Virus), avian and porcine influenza viruses, SARS-associated coronavirus, Nipah and Hendra viruses, and monkeypox virus.

The emergence of new viral infections often follows environmental, ecological and technological changes caused by human activities (Louz et al., 2005). Such activities may lead to an increased contact between humans and livestock on one hand, and animal hosts acting as reservoirs of zoonotic viruses on the other hand. Agricultural development, an increased exploitation of environmental resources, growth and increase in the mobility of the human population and trade and transportation of food and livestock, have been identified as important factors contributing to the introduction and spread of a number of new viruses in the human population.

Against this background the intensified use of viruses and their genetically modified variants as viral gene transfer vectors for biomedical research, experimental gene therapy and as live-vector vaccines is a cause for concern (reviewed by Louz et al., 2005).

### 7.4 Future safety prospects

There are now a number of examples that unwanted characteristics of poxvirus vectors can be modified or excluded by targeted mutagenesis, homologous recombination and reverse genetics (Najera et al., 2006; Chakroudi et al., 2005). But the safety benefits of these approaches can only be taken out when we have clarified putative GMV characteristics and adverse effect issues within the categories “known unknowns” as well as “unknown unknowns”.

## 8. The normative challenge by the concepts of harm and unwanted ecological consequences

In previous sections we have described several potential harms to the environment and discussed the relevance for ERA and risk management strategies. We will here discuss concepts and definitions related to harms and hazards in the context of legislative frameworks, and we will argue that for descriptive as well as for normative purposes, biological, ecological and ethical terms are needed for identification of unwanted harm and unwanted ecological consequences..

Endpoints of any risk assessment and risk management are always connected to the regulative framework. Article 1 of the Cartagena Protocol specifies that the entire objective of the document is to protect and conserve biodiversity according to a precautionary

approach. In the EU directive 2001/18/EC, it is stated that the applicant must submit a notification including an environmental risk assessment that considers direct and indirect effects, immediate and delayed effects, as well as potential cumulative and long term effects due to interaction with other GMOs and the environment. Harms to human and animal health are aspects that need to be characterised broadly. These include consideration of direct effects, e.g. development of disease, and indirect effects that may have a more complex nature; i.e. altered susceptibility to disease, welfare aspects and harm in social and ethical terms. With regard to harm to the environment, potential effects include the influence on and interactions with all organisms in the environment, and may be direct or indirect. Direct effects concern biological impacts on organisms, while indirect effects concern effects on animal health, contamination of wild gene pools or alterations in ecological relationships.

The legislative framework leaves many questions about what is to be treated as harm, which is a concept that both is descriptive and normative. For example, Raybould points to a central normative problem in the relationship between risk research and risk assessment of GM crops, and argues that the problem formulation (step 1) strongly depends on the respective stakeholder interests with regard to the environment and the goods and processes that need to be protected (Raybould, 2006). Moreover it is not only step 1 in environmental risk assessment but also step 5 that is a normative issue (Hill, 2005), hence risk assessments include value judgements both with regard to consequences that should be avoided and the process of risk characterisation. These choices are most often made before the risk assessment has been initiated. Accordingly, the question of harm is correlated to conception of human welfare and how to maintain and preserve nature and biodiversity. What is to count as serious harm to nature is based on contestable value judgments. There are for example distinct philosophical differences between giving priority to protection of human interests, i.e. anthropocentrism, versus preservation of ecosystems, i.e. biocentrism and ecocentrism (Dobson, 1998; Westra, 1998). In an anthropocentric context, the environment is protected to promote human well-being, as recreation purposes, or seen as a source for gaining new knowledge, assuming ecosystems to contain unknown information. Moreover, biodiversity centres represent valuable genetic pools for future agricultural and medicinal development. Hence, human interests provide a powerful set of motives for protecting the environment against activities that may have severe consequences (i.e. reduced biodiversity) for present and future generations.

Biocentrics and eccocentrics emphasize the need for a change from the anthropocentric exploitation of the environment towards a greater respect for the integrity of the animals and the environment. Biocentrics argue that as humans, we must provide rights to species and habitats and hence it is our duty to respect their integrity (Regan, 1980). An ecocentric approach involves more than consideration of impacts on human welfare and sustainability, and focuses on changes in both the biotic and the abiotic environment, as for instance effects on soil, water and air. Ecocentric approaches are not only aware of integrity of the animals and the environment, but do also acknowledge ecosystem integrity. In this context, preservation and protection of biological, ecological and genetic processes are necessary, irrespective of the instrumental value to humans. Application and release of GMVs can then be justified if it promotes the welfare of the ecosystem, or

when it protects or adds to the diversity of the species in the community. This ideology differs with regard to value commitments and factual beliefs from anthropocentric GMV governance. Hence, how we approach the environment and the values we put on the environment may also affect the frames and approaches chosen in environmental risk assessment and management.

### 8.1 Problem formulation processes for involvement of normative issues

To take into account the normative issues Nelson and Banker (2007) have suggested that step 1 in the environmental risk assessment need to be developed into a "Problem Formulation". Scientists in support of the ecological approach to risk assessment of GM crops (see section 5.1) have been involved in developing the problem formulation and option assessment (PFOA) tool, that is based on stocktaking exercises, stakeholder consultation and broader public participation procedures. The PFOA has been used in developing countries with the intention to improve the ERA and as a technology assessment tool and entails involvement of not only scientists but also the public in identification of the problem to be analysed. Various types of knowledge held by the public in general (local knowledge) as well as knowledge held by different interest groups and affected parties are here seen as valuable insights that may help to critically broaden the scope of the risk assessment and are also considered important in identification of protection goals. Moreover, local knowledge is also highly relevant with regard to development of risk management strategies and can give valuable insight with regard to local conditions relevant for risk associated research and in monitoring activities.

By transforming ERA into a problem formulation process we would like to add that besides including local knowledge there is also a need for broader scientific expertise to ensure a thorough assessment of the GMV in question. By extending the scientific experts involved from molecular biologists and virologists, to include ecologists, biologists as well as ethicists and sociologists, more diverse approaches to problem formulation processes can be ensured. Involvement of different scientific disciplines will also be crucial in elaboration of normative issues, in identification of protection goals and in development of management strategies. The scientists involved should share their opinions with the public as well as relevant political authorities.

## 9. Precautionary principle and precautionary approaches

We have discussed how risk assessment and management strategies are developed within particular frameworks, including normative standards and preferences regarding our relation to the natural environment and the preservation/promotion of the environment (see section 5 and 8). In such cases, decision-makers have to make decisions that will include the challenging issue of how to handle uncertainties. We consider that the uncertainties involved with GMVs entail that the precautionary principle needs to be employed (Myhr & Traavik, 2007).

The precautionary principle is a normative principle for making practical decisions under conditions of scientific uncertainty, and provides a general approach to environmental and health protection (EC, 2000). The actual content of the precautionary principle and the

practical implications of its implementation in policy issues are however controversial (Foster et al., 2000). Several versions of the principle, ranging from ecocentric to anthropocentric, and from risk-adverse to risk-taking positions have been put forward (Raffensperger & Tickner, 1999). Here we would like to acknowledge the version of the principle that can be found in the 1990 Bergen Declaration on Sustainable Development:

"In order to achieve sustainable development, policies must be based on the precautionary principle. Environmental measures must anticipate, prevent and attack causes of environmental degradation. Where there are threats of serious or irreversible damage, lack of full scientific certainty should not be used as a reason for postponing measures to prevent environmental degradation."

What we appreciate with this version is the commitment to anticipate, prevent and attack causes of environmental degradation. We also acknowledge the passive voice in the following part of this version of the principle; "lack of full scientific certainty should not be used as a reason for postponing measures to prevent environmental degradation", and see that this entails lack of requirement of action. However, this version of the principle connects the importance of taking caution in innovation with the achievement of sustainable development, and is active in nature by stating that in practice its application demands anticipation and prevention of harms. For GMVs the present lack of knowledge and the uncertainties with regard to environmental effects as for example with regard to potential recombinational events, non-target effects and transboundary spread entails the necessity to initiate targeted research, in line with a precautionary approach to decision-making. In this sense, it can be argued that this version of the principle extends from being a formulaic decision-making rule to also include an approach to include scientific activity in decision-making

The important elements of what represents a precautionary approach to decision-making are (Wickson et al., 2011):

1. The use of scientific research that is broadly framed, interdisciplinary, able to consider indirect causal mechanisms, and contributory to a lifecycle approach to analysis.
2. A recognition of the limitations of this scientific knowledge and a willingness to expose the knowledge to critical reflection and 'extended peer review', particularly so as to create transparency about embedded choices and assumptions.
3. A commitment to reducing uncertainties and minimising surprises generated by ignorance through vigilance and ongoing research and monitoring.
4. A transparent handling of ambiguity and indeterminacy through interdisciplinary approaches and broadly based public participation. This handling includes the consideration and implementation of a range of socio-technical alternatives and policy options.

## 10. Conclusion

So far no GMVs have been thoroughly risk assessed from an environmental point of view. Risk assessments have focused on unintended effects of the vaccine arising in the vaccinated individuals, or in individuals of the same species that are infected by virus shed from vaccinated individuals. Here we have elaborated that risk assessment and management

strategies need to be connected, and that a precautionary approach needs to be employed to GMVs.

A precautionary approach to GMVs includes initiation of risk-associated research with the intention to advance specific hypotheses about GMV specific harm and hazard assessment and management endpoints. Investigation of potential adverse effects and preventive measures needs to be initiated according to research and policy agendas encouraging broad and long-term thinking that supports precautionary actions. We consider that problem formulation processes represent a good approach for how to broaden the involvement of expertise and knowledge, and for identification of normative issues. As illustrated in section 8, risk management endpoints are dependent on both scientific and normative aspects, hence how nature, how harm and environmental damage are identified can influence the objectives of environmental risk management and thereby be used to identify specific targets for protection. These endpoints are also dependent on the objectives of environmental risk assessment research. After identification of endpoints, specific hypotheses for characterization of risk can be developed which enables targeted research to be carried out, where models are used and data are collected with the purpose of testing the risk hypotheses. We have in 4.5 briefly described some of the research that has been carried out in our own institution. We would like to stress that such approaches represent a good platform for research initiatives in other countries. Such risk-associated research can include questions presented in section 6.1. Accordingly, the assessment and management of potential adverse effects must include conception of the ecological background as well as normative discussions with regard to endpoints. Approaches that take this into account may secure that the final stages have a broad basis for decision-making: both with regard to representation and involvement of ecological aspects and ethical values.

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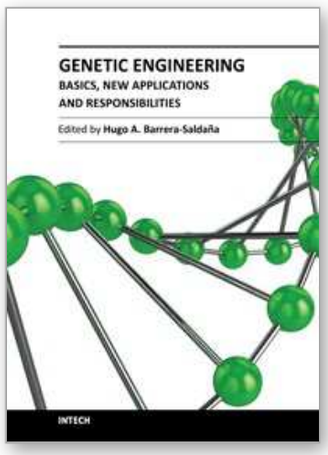


**Genetic Engineering - Basics, New Applications and Responsibilities**

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Leading scientists from different countries around the world contributed valuable essays on the basic applications and safety, as well as the ethical and moral considerations, of the powerful genetic engineering tools now available for modifying the molecules, pathways, and phenotypes of species of agricultural, industrial and even medical importance. After three decades of perfecting such tools, we now see a refined technology, surprisingly unexpected applications, and matured guidelines to avoid unintentional damage to our and other species, as well as the environment, while trying to contribute to solve the biological, medical and technical challenges of society and industry. Chapters on thermo-stabilization of luciferase, engineering of the phenylpropanoid pathway in a species of high demand for the paper industry, more efficient regeneration of transgenic soybean, viral resistant plants, and a novel approach for rapidly screening properties of newly discovered animal growth hormones, illustrate the state-of-the-art science and technology of genetic engineering, but also serve to raise public awareness of the pros and cons that this young scientific discipline has to offer to mankind.



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