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National Research Programme NRP 59 “Benefits and Risks
of the Deliberate Release of Genetically Modified Plants”:
Review of International Literature

Medical Issues Related to Genetically
Modified Plants of Relevance to Switzerland

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Aim of the three literature reviews

The National Research Programme “Benefits and Risks of the Deliberate Release of Genetically Modified Plants (NRP 59)” consists of four main areas of interest:

1. Plant biotechnology and the environment
2. Social, economic and political aspects
3. Risk-assessment, risk-management and decision-making processes
4. Synthesis and overview studies

It was neither in the capacity nor in the scope of NRP 59 to duplicate the many studies on benefits and risks associated with genetically modified plants (GMP) that have been carried out in other parts of the world. On the other hand, it may be possible to distil relevant and valuable scientific data from the results of such studies that could help to shape future research and decision-making processes specifically tailored for Switzerland. In the frame of focus point IV, three overview studies were therefore compiled by members of the Steering Committee of NRP 59 that evaluate on an international scale existing research and knowledge on topics that are of direct relevance to the central themes of NRP 59.

In the volume *“Medical issues related to genetically modified plants of relevance to Switzerland”* Karin Hoffmann-Sommergruber and Karoline Dorsch-Häsler provide an extensive overview of health-related risks and benefits of GM plants.

In the volume *“Genetically modified crop production: social sciences, agricultural economics, and costs and benefits of coexistence”*, Joachim Scholderer and Wim Verbeke assembled valuable insight obtained by screening literature databases and research/project portals, and through direct contacts with key researchers in the different areas.

In a comprehensive third volume entitled *“Synthesis and overview studies to evaluate existing research and knowledge on biological issues on GM plants of relevance to Swiss environments”*, Jeremy Sweet and Detlef Bartsch compiled information resulting from close to one thousand scientific publications relating to biological and environmental issues on GMP.

The chapters in this volume will not only be useful to a readership that is familiar with the biological, environmental, political, socio- and agro-economical aspects of GMP, it will also provide newcomers to the field with an in-depth introduction into a range of specialised topics that are relevant to this complex area.

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1. Introduction

Based on the original implementation plan of NRP 59 (National Research Programme 59), projects were invited to investigate the benefits and risks of genetically modified plants (GM plants). Therefore, aspects on the performance and functionality of GM plants as well as their influence on ecological systems and human health should be addressed. While Modules 1-3 focus on individual topics: related to environmental and health aspects (Module 1), political, social and economic aspects (Module 2) and risk-assessment, risk-management, and decision-making processes (Module 3), Module 4 is dedicated to synthesis and overview studies. Furthermore, Module 4 evaluates national and international ongoing research and knowledge with particular emphasis on data relevant for Switzerland. These syntheses should include a detailed analysis of the status quo, a summary of the actual state of the art technology and they should aim at providing information and advice for the Swiss politicians and the public.

The mandate of the NRP 59 was to provide the Swiss Federal Council by the end of 2009 with an Intermediate Report based on the first relevant results emerging from the research projects accompanied by the first synthesis studies. This information should contribute to a science-based political debate at the end of the moratorium on GM plants in 2010, which in fact was prolonged nonetheless for another three years.

However, since the project proposals submitted for Module 4 did not fulfill the expectations, the executive office decided to invite members of the Steering Committee and experts in the field to compile synthesis and overview studies on international and national research in the field which would represent valuable information also relevant to the Swiss economic and social situation and would assist in future decision making policy. These Module 4 overview studies were updated in 2011 in order to provide new information necessary for the final synthesis report of NRP 59.

The present report deals with health-related risks and benefits of GM plants. These health-related aspects comprise information on unanticipated adverse effects on humans and animals, how to prevent potential toxic and allergic reactions and what is currently known in order to develop an effective risk management strategy. In addition, the potential of GM plants for health-related purposes in Switzerland is evaluated, in particular GM-pharmaceutical plants and products thereof, e.g. vaccines or antibodies.

1.1 Brief Overview on GM Crops

The early GM crop-plants were designed to tolerate either pesticides and/or herbicides or to display resistance against insect or virus attacks. These plants do not exhibit substantial changes in their composition and they are called **GM plants of the first generation**. **Second generation GM plants** are modified regarding their output traits: *increased content of valuable components (amino acids, fatty acids, vitamins, etc.), an improved availability of nutrients or a lower concentration of undesirable substances (e.g. phytate, lignin, allergenic substances, etc. (Flachowsky et al., 2007))*. High value molecules are produced in **third generation GM crop plants**, such as human or animal vaccines or antibodies or industrially applied molecules. Products from third generation plants include nutraceuticals such as the “golden rice”, plant made pharmaceuticals (PMPs) (Spök and Karner, 2001) or plant made industrials (PMIs) (Sten et al., 2004), which are not discussed further here. PMPs and PMIs are usually not meant for human consumption except for therapeutic purposes.

Worldwide, GM plants are grown for commercial purposes in 29 countries, on a total of 148 million hectares (ISAAA Report 2010)¹. A total of 10 crops for food and feed purposes are commercially grown, the major crops being maize, soybean, rapeseed and cotton. Registrations exist for 24 types of different plants (ISAAA report 2010 and Center for Environmental Risk assessment²). Up to now, the major research has been done on herbicide tolerance, insect resistance and agronomic properties, as demonstrated by Table 1, showing numbers of releases for the United States.

Table 1: Distribution of approved releases by phenotypic category in the US

Classification of use of crop	Number of releases
Herbicide tolerance	6429
Insect resistance	4768
Agronomic properties	4500
Other	1853
Marker gene	1721
Virus resistance	1515
Fungal resistance	1197
Bacterial resistance	217
Nematode resistance	145

Adapted from Information Systems for Biotechnology. The numbers include permits as well as notifications.³

In the European Union, a total of 31 events in 7 plants have been authorized. They include rapeseed (male sterility and herbicide tolerance) soybean (herbicide-tolerance), sugar beet (herbicide tolerance), maize (insect resistance, herbicide tolerance), potato (altered composition), cotton (herbicide tolerance) and carnation (modified color).⁴ Field trials with plants expressing new, high-grade products, i.e. second and third generation GM plants with so-called output-traits have taken place, but unfortunately, it is very difficult to obtain current data of the number of field releases of PMPs and PMIs in the US as well as in Europe.

Internationally, a number of **plant-made pharmaceuticals** are now in advanced stages of development; about 50 have reached the stage of clinical trials⁵. A selection is shown in the Table below. Some of them have actually been admitted by the relevant competent authorities. However, it is not sure whether all of these pharmaceuticals already in clinical trials will actually be admitted for commercialization. From 1995 until 2006 about 5 km² of land were used for trials with PMPs.

¹ <http://www.isaaa.org/> (accessed 8 December, 2011)

² http://cera-gmc.org/index.php?action=gmc_crop_database (accessed 8 December, 2011)

³ <http://www.isb.vt.edu> (accessed 9 December, 2011)

⁴ <http://www.gmo-compass.org/eng/gmo/db/> (accessed 8 December, 2011)

⁵ <http://www.molecularfarming.com/PMPs-and-PMIPs.html> (accessed 8 November, 2011)

Table 2: Plant-made pharmaceuticals in advanced stages of development

Plant	Product	Use	Company	Clinical trial status
Carrot suspension cells	Human glucocerebrosidase	Treatment of Gaucher's disease	Protalix Therapeutics Israel/Pfizer	Approved by US FDA, 1 May 2012
Maize	Gastric lipase	Treatment of Cystic fibrosis	Meristem Therapeutics, France	Phase II
Safflower	Insulin	Diabetes	SemBioSys, Canada	Phase I/II completed 2009
Tobacco	Antibody cancer vaccine	Non-Hodgkin-Lymphoma	Large Scale Biology USA	Phase II
Tobacco	Antibody	HIV prophylactic	Pharma-Planta consortium	Phase I started 7/2011
Tobacco	Virus-like particles	H5N1 vaccine	Medicago	Phase II completed
Tobacco	CaroRX	Caries prophylaxis Antibody	Planet Biotechnology USA	Phase II, approved for use in Europe but not marketed
Lemna (Duckweed)	Cytokine	Hepatitis C	Biolex Therapeutics	Phase 2B results 3/2011
Rice	Human lysozyme	Food supplement: anti-infection, anti-inflammatory	Ventria, USA	Advanced, already commercially available as food additive
Tobacco suspension cells	HN protein of Newcastle disease virus	Poultry vaccine	Dow Agro Sciences USA	Approved by USA but not marketed

Adapted from (Paul and Ma, 2011; Spök and Karner, 2008), and MolecularFarming.com⁶

1.2 State of the Art in Switzerland

In Switzerland, four events (one for soybean, three for maize) have been authorized for food and feed purposes, but not for planting as shown in Table 3. For another 6 soybean events, 20 maize events and one cotton event, applications have been submitted with their authorizations pending.

⁶ <http://www.molecularfarming.com/PMs-and-PMIPs.html> (accessed 8 November, 2011)

Table 3: Authorized of GM plants for food and feed in Switzerland

GM plant	Event Trait	Unique Identifier, Company	Purpose	Status Dossier
Soybean	MON 40-3-2 ("Roundup Ready") Herbicide tolerant	MON-Ø4Ø32-6 Monsanto	Food Feed	Authorized 31/10/2002
Maize	Bt176 Insect resistance (BT)	SYN-EV176-9 Syngenta	Food Feed	Authorized 06/01/1998
Maize	Bt11 Insect resistant and herbicide tolerant	SYN-BTØ11-1 Syngenta	Food Feed	Authorized 14/10/1998
Maize	MON810 ("MaisGard") Insect resistant	MON-Ø81Ø-6 Monsanto	Food Feed	Authorized 27/07/2000

Source: Federal Office of Public Health⁷

A study published in 2005 showed that there were a total of 93 research projects with transgenic plants in Switzerland.⁸ These projects can be allocated as follows:

- 41% basic research projects
- 18% basic research projects with relevant application aspects
- 22% biosafety projects
- 19% applied research projects aiming to improve agronomic traits of food crops

The latter projects deal with fungal resistance in wheat, nematode-resistance in potatoes and carrots and scab resistance in apples. Other trait-specific projects are not specifically relevant for Switzerland, such as the development of virus resistance of cassava and mung beans or the increase in yield and quality of proteins in cassava and rice. Unfortunately, actual numbers are not available, but it appears, that neither the number nor type of projects has changed much.

Pharma plants

Within the NRP 59, two projects involve pharma plants; in the first one, HIV- and Hepatitis C antibodies are produced in transplastomic tobacco plants (Project by Felix Kessler, University of Neuchatel, described in Chapter 4), while the second makes use of chloroplast transformants of *Chlamydomonas* (single cell algae) expressing bacterial antigens to be used as fish vaccines (Project by Michel Goldschmidt-Clermont, University of Geneva and Joachim Frey, University of Bern). In Switzerland there are also other groups working with pharma plants in basic research. However, the pharma industry is not conducting any research with pharma plants in Switzerland at the moment (P. Ahl-Goy, personal communication).

⁷ <http://www.bag.admin.ch/themen/lebensmittel/04858/04863/04883/index.html?lang=de>

⁸ http://www.forschung-leben.ch/ffl_de/assets/File/BioFokus/BioFokus70.pdf

2. Legislation, Regulation and Authorities

Regulations regarding safety issues of novel food (e.g. new varieties etc.) have to undergo far less stringent safety investigations as compared to GM food and feed. In general, for non-GM plants, comparability and substantial equivalence to the original cultivar should be proven, but no safety issue has to be investigated in other respects. In contrast, various international and national regulators have laid down strict guidelines on how to perform risk assessment of GM-food and feed. In the following, legislation and regulations from the EU/EFSA, Switzerland and Codex Alimentarius are presented.

2.1 European Union

The general principles for food regulations are laid down in *Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002*. There the general principles and requirements of food law are described, the European Food Safety Authority (EFSA) established and the procedures in matters of food safety summarized.

Authorization of GM food and feed, including the requirements for an application and risk assessment is described in the *Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed*. This regulation was last amended by *Regulation (EC) No 298/2008 of the European Parliament and of the Council of 11 March 2008 regarding the implementing powers conferred on the Commission*.

The European Union regulates cultivation of GM plants by *Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC*. The directive describes the criteria to be fulfilled before a genetically modified organism (GMO) can be released or placed on the market. Important points of this directive are the step-by-step approval process as well as the case-by-case assessment of the risk of the GMO to human/animal health and the environment. In addition, the requirements for a monitoring plan are laid down here. Additional regulations as well as the corresponding URLs for the regulations mentioned here are listed in the Annex.

Since the environmental risk assessment of GM plants is discussed in another Module 4 report (Sweet and Bartsch, 2012), this topic will not be discussed here any further.

A detailed description of the legal background for dealing with GM foods in the European Union can be found in the *Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO)*, which has been updated recently (EFSA, 2011). The “Europa-Institut” at the University of Zürich provides an updated and detailed catalogue of EU-regulations concerning foods.⁹

⁹ <http://www.eiz.uzh.ch/recht/verzeichnis-eu-lebensmittelrecht/>

2.2 EFSA

The European Food Safety Authority (EFSA), funded by EU budget, but independent from the European Commission, European Parliament and EU Member States provides scientific advice on existing and emerging risks regarding food and feed in close collaboration with national authorities and in open consultation with its stakeholders¹⁰. Apart from scientific statements by the EFSA Working Group, EFSA has elaborated a number of very comprehensive documents on various aspects of risk assessment of GM food and feed. Some of them are indicated at the appropriate site throughout the text.

2.3 Switzerland

Swiss law is to a large extent harmonized with regulations of the European Union and this holds also true for food regulation. Swiss laws and ordinances are usually adapted to EU regulations within a reasonable time.

In the Federal Constitution, Articles 97 (Protection of consumers) and 118 (Protection of health) regulate food safety in Switzerland.¹¹ More details are laid down by the *Federal Act on Foodstuffs (Bundesgesetz vom 9. Oktober 1992 über Lebensmittel und Gebrauchsgegenstände*, SR 117.02) and its subordinate ordinances.

The following ordinances specifically regulate genetically modified foods:

- *Ordinance on Genetically Modified Foodstuffs (Verordnung vom 23.11.2005 über gentechnisch veränderte Lebensmittel*, SR 817.022.51)
- *Ordinance on Labelling of Foodstuffs (Verordnung vom 23.11.2005 über die Kennzeichnung und Anpreisung von Lebensmitteln*, SR 817.022.21)
- *Ordinance on Foods (Lebensmittel- und Gebrauchsgegenständeverordnung vom 23.11.2005*, SR 817.02).

The office responsible for food safety and human health is the Federal Office of Public Health FOPH. The various regulations relating to foodstuffs are summarized on the FOPH homepage.¹²

The *Federal Act relating to Non-human Gene Technology (GTL, Bundesgesetz über die Gentechnik im Ausserhumanbereich*¹³, SR 814.91, of 21.3.2003) sets the framework for dealing with genetically modified organisms in Switzerland. This law regulates the deliberate release of genetically modified organisms as well as their placing on the market as stated in Art. 27a GTL:

1 Genetically modified agricultural produce or additives may be manufactured, bred, imported, released experimentally or marketed only if the requirements of this law and, especially, those of the laws on gene technology, environmental protection, animal protection and food are fulfilled.

The accompanying *Ordinance on the release of organisms into the environment (Verordnung vom 10.9.2008 über den Umgang mit Organismen in der Umwelt*, SR 814.911) details the Gene Technology Law regarding release of GMOs into the environment. The legal re-

¹⁰ <http://www.efsa.europa.eu/en/aboutefsa.htm>

¹¹ All Swiss legal documents can be downloaded from the following URL:

<http://www.admin.ch/dokumentation/gesetz/index.html?lang=de>

¹² <http://www.bag.admin.ch/themen/lebensmittel/04858/04863/index.html?lang=de> and

<http://www.bag.admin.ch/themen/lebensmittel/04865/index.html?lang=de>

¹³ <http://www.bafu.admin.ch/biotechnologie/02618/index.html?lang=en>

quirements on environmental issues for marketing of GMOs as well as the links can be found on the biotechnology site of the Federal Office of the Environment.¹⁴

2.4 Codex Alimentarius

The Codex Alimentarius Commission (CAC) was established by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme.¹⁵ The Guideline for the Conduct of Food Safety Assessment of Foods derived from Recombinant-DNA Plants, was first adopted by the Commission in 2003 (Codex, 2003) with additional annexes being adopted in 2008 (Codex, 2009).¹⁶ This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. It addresses safety and nutritional aspects of foods consisting of, or derived from, plants that have a history of safe use as sources of food, and that have been modified by modern biotechnology to exhibit new or altered expression of traits (Guideline, Section 1,1). However, it does neither deal with safety aspects of animal foods nor with environmental risks.

An important step in the risk assessment process is the concept of substantial equivalence, which was developed by the Organisation for Economic Co-operation and Development (OECD) and published in 1993 the document *Safety evaluation of foods derived by modern biotechnology: concept and principles*.¹⁷ The concept of substantial equivalence is further discussed in Chapter 5.

2.5 Regulation of Third Generation GM Plants (Pharma Plants)

Regulation of third generation GM plants follows regulation of first generation GM plants in many respects. However, while first and second generation GM plants are primarily intended to be used as food and feed, third generation GM plants are designed for production either of pharmaceuticals or industrial molecules (Spök et al., 2008). Since PMPs are only in some specific cases planned for human consumption, they do not have to undergo the same risk assessment as GM plants. However, there is the risk of accidental exposure to and consumption of the GM plants by humans or wildlife. With regards to ecological risks, the possibility of non-intended cross-pollination or mixing with related plant species have to be assessed, especially in the case of deliberate release of pharma plants. In addition, the recombinant pharmaceutical active compound derived from pharma plants has to be analysed regarding its pharmacogenetics.

In the European Union, regulations of pharma plants are farther advanced than elsewhere. Discussions of these regulations can be found in various reviews (Sauter, 2005; Sparrow and Twyman, 2009; Spök et al., 2008). Pharma crops grown in open fields would require notification according to Directive 2001/18/EC, and EFSA would, according to Regulation 1829/2003, have a deciding role even if there were a proposal for non-food crops. GM crops grown in closed systems or as plant tissue culture can be regulated by the *Council Directive 98/81/EC of 26 October 1998 on the **contained use** of genetically modified micro-organisms*.

¹⁴ <http://www.bafu.admin.ch/biotechnologie/01760/index.html?lang=en>

¹⁵ <http://www.codexalimentarius.org/>

¹⁶ <http://www.fao.org/docrep/011/a1554e/a1554e00.htm>

¹⁷ <http://www.oecd.org/dataoecd/37/18/41036698.pdf>

EFSA has published the *Guidance Document of the Scientific Panel on Genetically Modified Organisms on the risk assessment of genetically modified plants used for non-food or non-feed purposes* in 2009 (EFSA, 2009). The document also contains a summary of the legal background for dealing with non-food plants such as pharma plants.

The only Guideline on plant-based pharmaceuticals already in force is the *Guideline on the quality of biological active substances produced by stable transgene expression in higher plants* by the **European Medicines Agency (EMA)**, in effect since February 1, 2009 following agreement by the Biologics Working Party (BWP) and adoption by the Committee for Medicinal Products for Human Use (CMPH).¹⁸ Further laws and regulations which have to be observed in the EU are listed therein.

The World Health Organisation (WHO) held an *Informal consultation on scientific basis for regulatory evaluation of candidate human vaccines from plants* in 2005 in Geneva. In that meeting it was concluded that the existing guidelines for the development, evaluation and use of vaccines were sufficient for the application of plant derived vaccines, even though some specific issues, such as plant containment would have to be addressed additionally.¹⁹

In contrast to the EMA Guideline, the Guidance for Industry *Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals* by the United States Food and Drug Administration (FDA) has been distributed for commenting purposes in 2002, but it has not been adopted as of December 2011.²⁰

In Switzerland, medicinal products are regulated by the *Federal Act of 15 December 2000 on Medicinal Products and Medical Devices (Therapeutic Products Act, Heilmittelgesetz, SR 812.21, as of 1 January 2009)* and the subordinate ordinances. Swissmedic, the Swiss Agency for Therapeutic Products, would most likely be the leading authority in case there was a trial. Up to now no specific legislation or guidance for pharmaceuticals produced in transgenic plants has been adopted. This is not too surprising, since no research project in Switzerland has been in an advanced stage close to a clinical trial.

¹⁸ http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003154.pdf

¹⁹ http://www.who.int/biologicals/Plant_Vaccine_Final_Mtg_Repor_Jan.2005.pdf

²⁰ <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm055424.pdf>
(accessed 9 December, 2011)

3. Benefits and Risks of GM Plants

The first generation of GM plants was primarily designed to improve the agronomic performance of the most important crops without addressing any issues regarding health related benefits. In contrast, the second generation of GM plants aimed at modified crops beneficial for health such as offering higher nutritional value e.g. higher unsaturated fatty acid, or added/elevated vitamin content. The possibility to down-regulate allergens in plants and to produce hypoallergenic fruits and vegetables also provides new options for the food allergic consumer. Furthermore, strategies to improve food processing procedures such as different starch composition in potatoes or reduced fungal contaminations were developed for GM plants.

Finally, exploiting GM plants as producers of transgenic pharmaceuticals offers several potential advantages compared to existing bacterial and animal cell expression systems. Several plant-derived pharmaceuticals have been developed and have already entered phase I and II clinical trials (Spök et al., 2008), one even phase III (Opar, 2011). For example, vaccines raised against hepatitis B, rabies and Norwalk virus have been produced in plants (Spök et al., 2008) and insulin and gastric lipase can be obtained from GM plants as well. At present phase I/II clinical trials have been terminated with insulin produced in safflower, demonstrating the quality, safety and pharmacological equivalence to insulin produced in *E. coli*²¹. For further details see also Table 2.

GM plants for nutritional purposes will have to be assessed for potential environmental and ecological risks. In the case of GM plants producing pharmaceuticals other risks related to occupational hazard have to be taken into account.

The safety assessment of GM plants should investigate any potential toxic effect due to the insertion of the transgene(s). Furthermore, increased potential allergenicity and possible changes in the immuno-modulatory activity have to be evaluated. In the case of allergenicity either the introduced gene products can de novo sensitize a predisposed individual or induce an allergic reaction due to cross reactivity to a homologous protein, or the intrinsic overall allergenicity of the host plant can be modified due to the insertion event.

While for toxicity studies threshold doses can be defined and state of the art animal studies performed, the situation is different for allergenicity testing. Threshold levels are not applicable in most cases and actual prevalence data of certain food allergies are lacking. However, new data are available on the in silico analysis of allergens which are useful for the allergenic risk assessment of newly expressed proteins. Whether animal models for allergenicity testing provide additional information will be discussed in this context. In the following, methods applied for traceability of the inserted gene products and studies based on substantial equivalence will be summarized, with special emphasis on the Swiss situation.

3.1 Health Benefits of GM Plants

As stated above GM plants can be generated to provide agronomic advantages and to offer better economic expectations ("1st generation GM plants"). Exploiting desoxyribonucleic acid (DNA) technology for developing crops with altered features made it possible to cross species barriers and to introduce new, wild-type and mutated genes to improve the agronomic performance. The original constructs aimed at increased resistance to pathogen attack and herbicide treatment, which should result in increased yield. The most important

²¹ <http://www.sembiosys.com/Products/Diabetes.aspx> (accessed 12 December, 2011)

herbicide resistant constructs are **glyphosate resistant** and glufosinate resistant plants which enable reduced total herbicide treatment and soil recovering during the recreation period (Halford and Shewry, 2000). GM plants expressing the *Bacillus thuringiensis* (Bt) gene, **Cry 1 protein**, are resistant to caterpillars; those usually cause great losses in the most important crops such as maize, sugar beet and potato.

In contrast to previous regimens of spraying the Bt formulation (*B. thuringiensis* spores producing Cry 1) several times during the season the protein (Cry protein; Bt toxin) is expressed within the plant at low levels and proved to be effective in reducing the damage created by the insects (Halford and Shewry, 2000). A recent study investigating the health condition of farmers in India and China identified a significant decrease in pesticide intoxication due to reduced exposure (Kouser and Qaim, 2011). In addition, it could be shown, that the Bt-maize was less contaminated with the mycotoxin Fumonisin. This mycotoxin created serious health problems in animals and humans, which were manifested at the sites of the pest attack due to infection by the *Fusarium sp.* fungus. Further aspects of Bt toxin related with health concerns are summarized and discussed in 4.2.

Although there has been a debate for more than a decade whether the 1st generation GM-crops reduce the burden of pesticide and herbicide treatment or not, reports have shown that the GM technology has the potential to reduce the inputs required in agricultural systems and has simplified the management practices depending on the agricultural system applied (Dunwell and Ford, 2005).

For food allergic consumers GM plants were designed which are low in allergen content. Around 2-3% of adults and 6-8% of children suffer from any type of food allergy in Europe and the US and develop either local or systemic life threatening reactions upon ingestion of the causative food.

Only around a dozen food sources are generally thought to account for 90% of the food allergy cases. In Europe, the most important food sources inducing a food allergic reaction in predisposed individuals are milk, egg, fish, peanut, tree nuts, celery, mustard, soybean, cereals, sesame seeds, lupine, crustaceans, and mollusks. Various fruits and vegetables also have allergenic potential (Asero et al., 2007). For some of these foods the relevance seems to be restricted to certain geographic regions and dietary habits.

Furthermore, out of hundreds of proteins present in the daily diet, only a restricted number of non-toxic proteins are able to induce the production of specific IgE antibodies and trigger an allergic reaction. At present around 200 different sequences encoding food allergens have been identified.²² The Pfam database version 23.0 comprises around 10,340 identified families of proteins with similar three-dimensional domains.²³ Among those, only 27 protein families/superfamilies were found having allergenic members, which accounted for more than 65% of all plant food allergens (Breiteneder and Mills, 2005; Radauer et al., 2008). Thus, the repertoire of allergenic proteins identified is small compared to the vast array of different proteins found in biology and indicates that conserved structures and biological activities play a role in determining or promoting allergenic properties of proteins. Therefore, attempts are currently made to collect and identify protein motifs which might be relevant for allergenic activity (Stadler and Stadler, 2003). Due to the limited number of food allergens, it seems feasible to address individual allergenic proteins in order to obtain low allergenic food alternatives.

²² <http://www.allergen.org/Allergen.aspx>

²³ <http://pfam.sanger.ac.uk/>

Genetic modification technology was applied to down-regulate levels of allergens in plant foods to obtain **low allergen alternatives**. Single-site mutagenesis of two IgE binding peptides of the soybean allergen, Gly m Bd 30 kDa has been proven to be effective in producing a hypoallergenic soybean protein (Helm et al., 2000). Single amino acid exchanges were also performed in the major apple allergen, Mal d 1, and the final Mal d 1 mutant showed much lower allergenic activity by in vitro (Dodo et al., 2008) as well as in vivo testing compared to the wild-type Mal d 1 (Ma et al., 2006). An alternative approach was taken using antisense RNA for the 14 kDa and 16 kDa allergenic proteins in rice, which repressed the allergen gene expression in maturing seeds and resulted in reduced allergenicity of this rice cultivar (Nakamura and Matsuda, 1996; Tada et al., 1996). The same method was applied for soybean, targeting the Gly m Bd 30 kDa gene and after successful transformation, this protein could no longer be detected (Herman, 2003). Further examples were shown for down-regulating the major apple allergen, Mal d 1 (Gilissen et al., 2005). The GM-apple plantlets were virtually free of Mal d 1 as shown by immunoblots and skin prick tests. Recently, transgenic tomato fruits suppressing expression of two known tomato allergens, profilin (Le et al., 2006) and non-specific lipid transfer protein (Lorenz et al., 2006), were obtained by applying the double-stranded RNA interference (dsRNAi) technology. Also in peanut, the most relevant allergen, Ara h 2 was successfully down regulated by the RNAi strategy (Dodo, Konan et al. 2008).

Although these examples are promising and represent a choice for the allergic consumer, it is important to note that this method only targets single proteins/allergens, and while one allergen is suppressed or expressed at very low levels, other known allergens are still expressed in the plant food.

In addition, it has to be stated that the acceptance of these hypoallergenic GM plants is rather low – even in the target consumer group – as evaluated by a survey performed in 3 European countries (Miles et al., 2005). At present these approaches are of scientific value and highlight the possibility to down-regulate individual allergen levels through genetic modification. However, the impact on the remaining allergenicity of the whole food remains to be demonstrated on a case-by-case basis.

Celiac disease, another type of allergic disorder is caused by gluten, the protein fraction (containing gliadins) from wheat and related cereals in genetically predisposed individuals, and it affects around 1% of the population from Western Europe. This adverse reaction upon intake of gliadin proteins from cereal products fuels the chronic inflammation of the bowel and causes malabsorption. Therefore, for these patients the method of choice is to avoid cereals and products thereof. In order to offer these consumers at risk less immunogenic alternatives, studies have been undertaken to identify the relevant immunogenic epitopes in gliadins and to select natural forms with no or less immunogenic activity (van Herpen et al., 2006). Alternatively, the RNAi technology was applied to silence either the alpha-gliadin (Becker et al., 2006) or the gamma-gliadin genes (Gil-Humanes et al., 2008). However, it has to be noted that down-regulation of the expression of the gliadins also affects the processing quality of wheat grains (Shewry, 2009).

Apart from the possibility to remove proteins with unwanted effects from (plant) food by the use of GM-technology, there is also the option to design “**functional foods**”. Any health-promoting or disease-preventing property of food beyond the pure nutritional function is summarized by this definition and includes e.g. the addition of vitamins or fibers with an identified health benefit and being superior to the wild-type organism. Over the last decade considerable progress has been made on elucidating biosynthetic pathways for relevant human health components. By the use of GM technology the production and up-regulation of vitamins, novel long-chain polyunsaturated fatty acids, increased flavonoids and carotenoids in crops has become feasible (Davies, 2007).

While the 1st generation of GM plants with disease resistance genes is already commercialized, it is to be expected that the established 2nd generation of GM crops with various nutritional benefits will be released for agricultural use within the next decade. Prerequisites for production or accumulation of metabolites of interest in the plant are the availability of DNA sequences encoding the biosynthetic enzymes or regulatory factors and effective GM techniques. The RNA interference (RNAi) has proven to be an effective and reliable approach to inhibit gene activity for one of the biosynthetic enzymes. In contrast, for up-regulating expression of certain genes of interest, introduction of transcription factors is the method of choice. Among the metabolic engineering approaches in plants (Hindley et al.), expression of vitamin A, B (folate), C and E has been addressed in a number of studies. The best known is the “Golden Rice” project, the attempt to introduce provitamin A into a staple food, rice, which was developed by Ingo Potrykus (Prof. em. from the ETH Zurich). It is well known that due to an unbalanced diet in the Asian area millions of children and adults suffer from blindness. By supplementing the vitamin uptake via GM-rice, this vitamin A deficiency could be tackled. Within the last decade I. Potrykus and P. Beyer succeeded in introducing a functional provitamin A pathway into the rice endosperm (Burkhardt et al., 1997; Enserink, 2008; Ye et al., 2000). The resulting GM plants are distinct from the wild-type plants by a yellow-colored endosperm containing provitamin A and other terpenoids of nutritional value (Potrykus, 2001). This non-profit project is dedicated to support the population of the developing countries and aims at making this technology freely available for local farming. Since the proof of concept is already delivered, the actual bio-availability of the vitamin has to be assessed in field trials in the forthcoming years (Al-Babili and Beyer, 2005). In addition, the required substantial equivalence, allergenicity and toxicology assessment will have to be undertaken in due course. In the meantime other similar approaches have been established in transgenic rice yielding higher carotenoid levels in the endosperm, e.g. up to 37 micro-grams per gram dry weight (Paine et al., 2005).

Expression of **vitamin E** (tocopherol and different types of tocotrienols) has been successfully up-regulated in GM plants, especially in seeds of oil crops (Davies, 2007). **Vitamin B9** (folate), a relevant factor for decreasing the risk of cardiovascular diseases and cancer also has been successfully over-expressed in tomato fruits, and **vitamin C** could be up-regulated in maize and lettuce (Davies, 2007).

Apart from vitamins, **minerals** such as iron, zinc, iodine, calcium, magnesium and selenium are an essential part of the human diet. In order to supplement low intake of minerals, GM approaches to increase amounts of minerals in food crops were established. Among those,

the increase of the absolute mineral content is not the only goal. Bioavailability of the individual minerals and identification of relevant compounds is another issue that can be approached by GM-technology. However, the current understanding of the relevant plant mineral processes is rather small and only a few GM-constructs have been developed (Davies, 2007).

Other metabolic engineering approaches for functional foods address the expression of isoflavonoids acting as phytoestrogens (for further information see (Davies, 2007). Several approaches have been set up to increase the production of **long-chain polyunsaturated fatty acids** in plants. Since the evidence of health benefit from polyunsaturated fatty acids is only shown for the animal-derived fatty acids, e.g. from fish oil (omega-3-long chain polyunsaturated fatty acids), experiments have been undertaken to transform oil seed plants (linseed, soybean and mustard) with the genes involved in the biosynthesis of omega-3-long chain polyunsaturated fatty acids (Chen et al., 2006). Soybean with an improved fatty acid composition has been approved as food and feed in the US, Canada and Mexico.²⁴

Finally, functional foods with a benefit for the gut health have been designed. By modifying the biosynthesis of **starch** in plants, fructans, i.e. fructose biopolymers can be over-expressed. Fructans cannot be digested by humans but are degraded by beneficial gut bacteria such as Lactobacilli and Bifidobacteria. In addition, fructans have been reported to facilitate mineral absorption and to prevent colon cancer via prebiotic and other mechanisms. Therefore, GM-crops such as sugar beets and potatoes have been developed with significant fructan expression in their tubers (Weyens et al., 2004). GM-potatoes with an altered starch composition leading to higher resistance towards digestion and thus promoting gut health were also developed (see Davies, 2007).

Using GM-technology, not only up-regulation of highly valuable nutrients in crops is achievable, but also down-regulation of undesired compounds, such as **toxins**. Cassava, for example, an African food crop, contains a high content of cyanogenic glucosides. Elimination of these unwanted toxins was achieved by the RNAi strategy (Jorgensen et al., 2005).

In summary, a number of feasibility studies have provided evidence of the improvement of the nutritional value of crops. However, as stated above, most of these crops have not yet been commercialized, and safety studies are needed to ensure that altering shared metabolic pathways by GM-technology does not have an impact on other important pathways.

Another issue to be addressed is the qualitative improvement of food by GM-technology relevant for food-industrial purposes without any health related benefit. For example, GM-potatoes with altered starch content were developed by the RNAi strategy by down-regulation of the starch synthetase, resulting in low amylase and high amylopectin potato tubers. This altered starch possesses superior features for the production of glue. The EU Commission has authorized the so-called Amflora potato, which has an improved starch composition, for cultivation and industrial use on 2 March 2010.²⁵

²⁴ http://cera-gmc.org/index.php?action=gm_crop_database&mode=ShowProd&data=DP-305423

²⁵ <http://www.transgen.de/zulassung/gvo/17.doku.html>

Table 4: Selected examples of GM plants with health benefit (extensive list can be found in Davies, 2007; EFSA, 2008; ILSI, 2004)

Plant/Species	Altered characteristics	Reference
Starch		
Potato	Fructan ↑	Van der Meer et al. 1994
Fatty acids		
Canola	+ ω-3 Fatty acid	ILSI 2004
Soybean	LC-PUFAs* ↑	Chen et al. 2006
Toxin		
Manihot	Cyanogenic glycoside ↓	Jørgensen et al. 2005
Vitamins		
Rice	provitamin A ↑ (Golden Rice)	Hoa et al. 2003
Canola	Carotenoids (including provitamin A) ↑	Ravanello et al. 2003
Thale cress	Tocopherols ↑	Cahoon et al. 2003
Maize	Tocopherols ↑	Cahoon et al. 2003
Tomato	Phytosterols ↑	Enfissi et al. 2005
Allergens		
Rice	Allergenic Protein ↓	ILSI 2004
Soybean	Immunodominant allergen ↓	ILSI 2004
Apple	Major allergen (Mal d 1) ↓	Gilissen et al. 2005
Tomato	Allergen (Lyc e 1) ↓	Le et al. 2006
Tomato	Allergen (Lyc e 3) ↓	Lorenz et al. 2006

* LC-PUFAs – light chain poly unsaturated fatty acids

3.2 Health Risks due to Inserted Genes and Genetic Modification

Adverse effects of GM plants can be toxic effects upon ingestion, or induction of an undesired immune response such as an allergic reaction or an immune-modulating activity. For toxicity studies threshold doses can be identified and state of the art animal studies are performed, since toxic substances affect all individuals exposed with only minor inter-individual differences in susceptibility. The mechanisms regarding **food intolerance** are not well understood, but in many cases, they represent the consequence of a defect in digestion or metabolism. For allergenicity testing the situation is different.

Food allergy is an adverse reaction to food, which represents an important public health problem and it may also affect patients with inhalant allergies who can cross-react to proteins in pollen or in food derived from GM plants.

The prevention and management of food allergy is a responsibility of governmental regulatory bodies as well as the agrofood industry. It is the responsibility of scientific risk assessment bodies and a prerequisite for marketing of novel foods and GM foods to assess the allergenicity of a given food (which has the capacity to induce an allergy) (Codex, 2003).

Sometimes minute amounts of a food that is well tolerated by the vast majority of the population can cause serious symptoms and death in allergic individuals. It is not the allergen, but the allergic person's abnormal reaction to the allergen that causes the adverse health effect. Food allergy can be caused by various immune mechanisms. However, IgE-mediated food allergy represents the main form of food allergy causing the most severe reactions and it is the only form causing life-threatening reactions. Up to now this IgE-mediated food allergy has been the focus in the **risk assessment of allergenicity of GMOs and novel foods** (2003).

When assessing allergenicity of novel food such as GM food/feed, the main issues that clearly have to be addressed are the allergenicity of the newly expressed protein(s) and the intrinsic allergenicity of the whole food/feed and derived products as an unintended effect of the genetic modification. Another issue to be taken into account is a possible increase in allergenicity via the intake/exposure of the GM food. An example of introducing a known allergen into a food where it did not exist before is the introduction of the 2S albumin, a Brazil nut allergen into soybean (Nordlee et al., 1996). Although this GM-soy was designed for animal feed exclusively, the increased allergenicity was verified and the project terminated due to this increased allergenic risk.

In order to increase disease resistance in plants, transgenic expression or up-regulation of **pathogenesis-related proteins** (PR-proteins, (van Loon and van Strien, 1999) such as chitinases, glucanases and thaumatin-like proteins has been tested (see also the project by Sauter within the NFP59). However, allergenic activity of proteins belonging to these PR-protein families and the potential increased allergenic risk of such GM plants has been identified (Hoffmann-Sommergruber, 2000). Clear guidelines have been set up for evaluating an increased risk of unintended exposure to potential food allergens by e.g. the Codex Alimentarius, WHO/FAO and EFSA (for further details see Chapter 5.2.). Among those, the bioinformatic approach, that is a search for significant sequence similarity to already known allergen encoding sequences, is a key element. Another important method is the use of allergic patients sera in several in vitro IgE antibody-binding assays to verify potential cross reactivity of the novel protein with known allergens. The limitation of this risk assessment is the lack of information on novel proteins or proteins previously not frequently consumed by humans (Goodman et al., 2008) (EFSA, 2010). The potential to induce allergic reactions cannot easily be predicted and will only be assessable after placing on the market.

Concerning possible **immuno-modulatory** effects of a transgenic product, the data are scarce. For example, adjuvants (substances that increase the immune response in a host when administered with an antigen) can be grouped into several classes such as CpG motifs of DNA, alum and bacterial toxins or toxin components. Depending on the administration regime of an adjuvant together with antigen(s), different types of immune responses can be induced in the host organisms which can be either of beneficial nature or causing adverse effects (Guy, 2007). At present, adjuvanticity is not commonly considered in the risk assessment of GMOs and further research in this area should help to clarify this issue.

In the context of risks of GM plants for human health, discussions have predominately focused on a few aspects. Among those, the various **Bt proteins** have been of public concern. The Bt proteins are rather unstable within the gut of vertebrates (with the exception of Cry 9c) and are degraded within a short time (Herman et al., 2003). Low protein stability contributes to an overall rather low potential allergenic activity (Astwood et al., 1996) and no Cry proteins were detected outside of the gut. In contrast, Bt toxins are stable upon proteolytic activation in the alkaline insect gut, bind to specific receptors present in the

midgut and exert a highly specific toxic activity on insects. So far no allergic reactions in humans have been reported and there is no relevant sequence similarity to known allergens identified. A considerable number of feeding studies have investigated the nutritional qualities of Bt crops including parameters such as digestibility, feed intake and health performance of the target animal. For example, Flachowsky et al. summarized 16 studies being performed to determine the effect of 1st generation GM plant feed on domestic animals (Flachowsky et al., 2007). No significant differences were observed, neither in the chemical analyses nor in the animal studies. The Technical University of Munich performed a feeding study with 36 cattle over a period of 25 months investigating effects of uptake of GM maize (MON 810) (Guertler et al., 2010; Meyer et al., 2009). The authors concluded that the feeding quality of the GM maize was equivalent to the isogenic maize and did not adversely affect the metabolism, health or performance of the cattle within the observation period. Cry 1Ab was found to be readily degraded in the digestive tract of cattle and no differences in the milk samples from GM maize-fed cows and control cows were identified (Meyer et al., 2009).

Other studies have claimed to prove adverse effects upon feeding with Bt crop such as differences in body weight and growth, hepatorenal toxicity (Seralini et al., 2007). However, by applying different statistical analyses contradicting results were identified (Hammond 2006). Upon evaluation, EFSA has so far not found any convincing evidence of an increased risk of a Bt crop and evaluated this study (and others) as not providing evidence of biological or toxicological relevance (Statement of the Scientific Panel on Genetically Modified Organisms on the analysis of data from a 90-day rat feeding study with MON 863 maize, European Food Safety Authority.²⁶ The panel concluded that *“different approaches are applied in the statistical analysis of data obtained from animal experiments and that there is a need for a harmonised approach for statistical analysis and interpretation of data”* in the area of safety assessment of crops.

Another matter of continuous debate is the uptake and potential transfer of **GM-derived DNA**. Based on the universal nature of DNA no difference is to be expected between wild-type plant-derived DNA and GM plant-derived DNA, respectively. DNA is part of the diet and accounts for 0.2 g/kg dry matter in plant-derived food (Beever and Kemp, 2000) and is expected to be digested. Of the total uptake of foreign DNA, the percentage of GM-derived DNA concentration is much lower. The majority of studies did not identify foreign DNA outside of the digestive tract of animals (Alexander et al., 2007). However, other studies were able to detect foreign DNA fragments in other tissues (Mazza et al., 2005; Sharma et al., 2006). In summary, the scientific community does not expect a health-related problem due to the uptake of foreign DNA and the presence of DNA fragments in animal tissues.

The possibility of a **horizontal gene transfer** from plants to gut bacteria has also been investigated, especially in the context of **antibiotic resistance, as marker genes** are frequently used as selection markers in 1st generation of GM plants. The potential transfer of antibiotic resistance genes would then contribute to multi-drug resistance. However, the likelihood of the transfer of an intact DNA gene sequence from plant tissue to bacteria is very low due to genetic incompatibilities and to barriers evolved in prokaryotes to suppress gene transfer (Brigulla and Wackernagel, 2010; de Vries and Wackernagel, 2005). Horizontal gene transfer could be demonstrated only under optimal laboratory conditions and not under natural conditions (de Vries et al., 2004).

With one exception, the class of antibiotics originally used as selection markers belong to antibiotics used for topical application and are not designed for oral administration. However, this is not a concern for Switzerland, since the Swiss Gene Technology Law from 20 March 2003 states in Art 6, para 1c: *“Genetically modified organisms do not contain gene*

²⁶ http://www.efsa.europa.eu/EFSA/Statement/GMO_statement_MON863,0.pdf

technologically inserted resistance genes to antibiotics used in human or veterinary medicine". The law applies only to GM plants developed after the law has come into force.

In order to meet the public concern, approaches identifying alternative selection markers replacing antibiotic resistance markers or omitting them at all have been set up (Goldstein et al., 2005; Zhang et al., 2006).

Gene transfer between a plant and a related pathogenic prokaryote is another possible risk. Potrykus and coworkers calculated the frequency of a horizontal gene transfer under optimal controlled conditions to be 6.3×10^{-2} which decreases to 2.0×10^{-17} under idealized natural conditions (Schlüter et al., 1995). Therefore, the authors concluded that the chance of a horizontal gene transfer is negligible.

Apart from assessing adverse effects due to a single-target GM-product, the overall altered composition within the GM plant also needs to be investigated for potential changes. This is the case especially in approaches where metabolic pathways important for more than one enzyme are targeted. Here the advanced profiling technologies such as proteomics or metabolomics will have to be applied to identify subtle changes in the overall composition. This topic is further discussed in Chapter 5.2.

3.3 Benefits and Risks of Pharma Plants

Plants have been an important source for preventing and healing diseases for thousands of years. However, following the introduction of GM plants, it took another ten years until in 1989 a murine antibody was expressed in tobacco (Hiatt et al., 1989), and in 1990 human serum albumin was produced in tobacco and potato (Sijmons et al., 1990; Twyman et al., 2005). Meanwhile, research has progressed rapidly in the field of so-called pharming, and a large number of publications and reviews have appeared on production of mammalian proteins in plants or plant cells (Paul and Ma, 2011; Rybicki, 2010; Spök and Karner, 2008; Yusibov et al., 2011).

Switzerland should be especially suited for producing GM plant pharmaceuticals due to its long and well-established experience with pharmaceuticals, including plant pharmaceuticals. At the moment, however, research is limited to academia; two projects have actually been performed within the NRP 59 program (see below).

In the EU, interest in plant pharming has been marked by setting up the so-called Pharma-Planta program as part of the 6th Framework Research program²⁷. The research program, a consortium of 39 scientists from 12 European countries and South Africa started in February 2004 and lasted for 5 years. With this program, the EU wanted to address the tremendous potential of plants being able to produce pharmaceutical proteins, but the researchers also wanted to develop robust risk assessment and risk management practices and collaborate with regulatory bodies to develop solid regulations. One aim of the program was to get the HIV-antibody 2G12 through the process of production in tobacco plants, through risk assessment and regulatory affairs up to the point of a clinical trial. This goal has been reached, albeit later than expected mainly due to regulatory obstacles and difficulties in securing a permit to produce the drug. The phase 1 clinical trial started in July 2011²⁸ and possibly because of the Pharma-Planta program the EMEA Guidance Document (see Chapter 3.5.) came into force in 2009. The 2G12 antibody has previously been shown to be safe in clinical trials when produced in Chinese Hamster Ovary (CHO) cells (Armbruster et

²⁷ <http://www.pharma-planta.net/>

²⁸ http://blogs.nature.com/news/2011/07/plantbased_drug.html

al., 2002). One Swiss Group participated in the Pharma-Planta project: Jean-Marc Neuhaus from the University of Neuchatel was involved in the production of tuberculosis antigens (Frutos et al., 2008).

For a number of reasons, there is great interest in using plants or plant cells as bioreactors for producing pharmaceuticals, such as vaccines, monoclonal antibodies, enzymes etc. The major advantage is the relatively low cost for large-scale production; the production cost also decreases if fewer steps are needed in down-stream processing. The number of steps depends on the concentration of the desired protein. Consequently, a careful choice of the plant tissue and of the specific expression technology is crucial. The other big advantage is the ease for large-scale production (in the case of plants even unlimited production) (Dunwell, 2005; Fischer et al., 2004; Kaiser, 2008; Paul and Ma, 2011; Sauter, 2005; Sparrow et al., 2007; Spök and Karner, 2008; Spök et al., 2008; Twyman et al., 2005).

On the other side, problems arise from the fact that there are differences regarding post-translational modification of mammalian proteins expressed in plants, even though the protein synthesis and folding pathways are highly conserved between animals and plants. The glycan structures of plant-derived mammalian glycoproteins differ from the native proteins. The glycan structure of a protein can play an important role in the protein stability, solubility, biological activity and immunogenicity. Consequently a number of strategies have to be applied to humanize the glycan structure in plant-made mammalian proteins. Other difficulties (list incomplete) are posed by the long production time and regulatory compliance (in the case of whole plants), biosafety and construct-size limitations (in the case of virus-infected plants) (Paul and Ma, 2011; Spök and Karner, 2008).

Plant cell cultures

The use of plant cell cultures (suspension and callus cell cultures, root and hairy root cultures) offers a number of advantages over whole plants (Dunwell, 2005; Dunwell and Ford, 2005; Hellwig et al., 2004).

Plant cell systems need shorter development times than whole plants, in some cases (root and hairy root cultures), the expressed proteins are secreted, there are much less variations in product yield and quality, and the application of the Good Manufacturing Practice (GMP) rules is much easier. The tissue culture media are simple synthetic media and there are fewer undesired by-products like fibers, phenolics, waxes etc. than in whole plants. Quite a large number of proteins of medical relevance have been produced, mostly in tobacco cell suspension cultures, but also in rice cells, soybean and carrot cells. Recently, the entire mammalian glycan synthetic pathway has been introduced into tobacco cells, allowing production of large quantities of a very uniform population of animal proteins in plant cells with glycan structures identical to those produced in mammalian cells (Castilho et al., 2010).

However, there are a number of disadvantages with cell cultures, such as slow growth rates, gene silencing, in some cases relatively low protein yields, lower stability of the produced proteins, or problems with cell clumping. These problems are being worked at and can also be solved by improving fermenter design, optimization of the nutrient supply, selection of cell lines taking into consideration the product formation, growth characteristics but also genetic stability.

The United States Department of Agriculture (USDA) has licensed a poultry vaccine against Newcastle disease virus produced in tobacco cell culture by Dow AgroSciences. The company objective was a proof of concept of their plant cells to pave the regulatory pathway and show that the strict standards required could be met, but there was no plan to commercialize the vaccine, and in fact the product is not being sold as of October 2011.

prGCD, a recombinant glucocerebrosidase (GCD) enzyme for treatment of the rare, inheritable Gaucher disease, is most likely the first plant-made pharmaceutical for human use which is going to be on the market. It is produced in carrot cells and was developed by Protalix Biotherapeutics (Opar, 2011). This drug has gone through clinical trials and it has been approved by the US Food and Drug Administration (FDA) as of 1 May 2012.²⁹

Transient expression systems

In the so-called transient expression system, the new gene is inserted into a non-GM plant by a vector. The big advantage of the system is that it can be developed much faster than a stable plant cell line. Relatively large amounts of protein can be produced over a few days (up to 40% of the protein product quantity relative to total soluble protein yield) (Davies, 2010b). An edible rabies vaccine was produced in spinach leaves using a tobacco mosaic virus-based hybrid vector, and a vaccine for avian influenza was produced by transient expression of hemagglutinin in tobacco plants. Both vaccines have been tested in humans in clinical phase I trials, and they proved to be safe and well tolerated (Yusibov et al., 2011). The system is also used for commercial production of the trypsin inhibitor aprotinin, a reagent that is distributed through Sigma. The sales advantage of plant-made aprotinin is the production in the absence of animal serum ("animal-free"). The use of animal tissue culture or other animal research products, especially bovine products, has become very difficult and expensive, due to possible contaminations with human or animal pathogens, and especially with BSE. Therefore, it is expected that the demand of products made in plants will increase in the future for safety reasons.

Transplastomic plants

Transplastomic plants, i.e. GM plants in which the new genes have been inserted into chloroplast DNA, offer several advantages (Bock, 2007; Lössl and Waheed, 2011; Ma et al., 2003; Ruf et al., 2007): The probability of transmission of chloroplasts by pollen is extremely low, and consequently transplastomic cells serve as a biological containment system. Further advantages are the site-specific integration of the desired gene, high-level foreign protein expression, and marker-free plants (by using the cre-lox system). Downstream-processing should be relatively simple and this system is thus cost-effective (Ma et al., 2003). In one of the NRP 59 projects, Felix Kessler has used transplastomic tobacco plants and has successfully produced the HIV p24 and HCV core proteins. They attached the foreign protein gene to the plastoglobulin gene. Plastoglobulins are highly conserved structural proteins present at the surface of plastoglobules, lipid droplets present in leaf chloroplasts, which float on top of an extract and can very easily be collected. The HIV antibody was thus purified to near homogeneity. These antibodies will eventually be tested in immunization experiments in mice.

Food crops

Many food crops offer a regulatory advantage due to their GRAS (**G**enerally **R**ecognized **A**s **S**afe) status stemming from long experience with the safety of these plants (Sparrow et al., 2007; Yusibov et al., 2011). A number of these crops have been used to express pharmaceutical proteins either in leaves, tubers, fruits or seeds, each offering specific advantage (see Table 5), and some of the products have gone through clinical trials. There is growing evidence that plant-based oral vaccines are not only feasible, but also effective (Rybicki, 2010; Tacket, 2009). Trials have also been performed with so-called edible vaccines (e.g. spinach leaves or bananas, potatoes, rice). The hepatitis B surface antigen has been expressed in potato and lettuce, and rabies virus GP/NP was produced in spinach.

²⁹ <http://www.nature.com/news/drug-making-plant-blooms-1.10604> (accessed 6 June, 2012)

The vaccines have undergone phase I clinical testing and an immune response was demonstrated (Yusibov et al., 2011). The success of these vaccines, however, is hampered by a number of regulatory problems, such as dosage, quality control and storage. Due to a couple of incidents in the United States where pharma crops were mixed with food crops, the use of food crops for the production of pharmaceutical proteins has drastically gone down and non-food crops are favored (Rybicki, 2010).

Overview of plants and production systems

A large number of plants and plant cell systems have been tested for production of pharmaceutical proteins, and not all can be described here extensively. Advantages and disadvantages of the various types of plants and plant systems are summarized in Table 5 below.

Table 5: Advantages and disadvantages of different plants and plant cells for production of pharmaceuticals

Crop species	Advantage	Disadvantage
Non-food crops Tobacco, falseflax (<i>Carmelina sativa</i>), safflower (<i>Carthamus tinctorius</i>)	Low risk of inadvertent mix with material for human consumption; Tobacco: well-established methodologies; protein targeting to secretory pathway	Alkaloids in tobacco leaves
Seed crops Maize, rice, parley, oilseed, soybean, safflower	Suitable for small or large/complex proteins; Easy long-term seed storage and expressed proteins; Very large amounts; Inexpensive	Starch complicates recovery; Plant must go through flowering cycle – risk of pollen transfer to other plants; Seed spillage and spread
Vegetable crops Potato, carrot	Good bio-containment	Alkaloids in potato leaves; Necessity to cook potatoes for consumption
Fruit/green leaf crops Alfalfa, lettuce, banana, tomato	Edible part can be consumed uncooked	Alkaloids in tobacco leaves; Low expression levels; Starch complicates recovery; Need for immediate processing; Proteins often unstable; Impossible to maintain consistency
Other species Lemna, <i>Chlamydomonas reinhardtii</i>	Very low risk of inadvertent introduction into food chain; Good containment possible	Scalability
Plant cell cultures	Secretion of protein into medium; Containment easily maintained; Regulatory compliance (GMP); Simple culture medium, no animal serum needed	Cost

Table adapted from Fischer et al., 2004; Sparrow et al., 2007.

In many respects, transgenic plants or plant cells used as protein expression systems are as good or even better compared to other protein expression systems currently used. A comparison between the various systems and their advantages and disadvantages is shown in the following table.

Table 6: Comparison of different protein expression systems

System	Production timescale	Scale-up capacity	Contamination risks	Product quality	Glycosylation	Overall cost
Bacteria	Short	High	endotoxins	Low	None	Low
Yeast	Medium	High	Low risk	Medium	Incorrect	Medium
Mammalian cell culture	Long	Very low	Viruses, prions and oncogenic sequences	Very high	Correct	High
Transgenic animals	Very long	Low	Viruses, prions and oncogenic sequences	Very high	Correct	High
Plant cell culture	Medium	Medium	Low risk	High	Minor differences or none	Medium
Transgenic plants	Long	Very high	Low risk	High	Minor differences	Very low

Table adapted from Sparrow et al., 2007.

The low cost of pharmaceutical protein production in plant cells has been contested, it was claimed, it would be higher than generally assumed (Spök and Karner, 2008). For Switzerland, production of proteins in plant cells is most likely the way to go, at least in the near future, because it would probably be very difficult to find a location in Switzerland for planting pharma plants, unless this would be done in a greenhouse at much higher cost and yielding limited quantities.

Risks associated with pharma plants and plant-made pharmaceuticals

The following major risks have to be considered that pharma plants can cause to human health (Sparrow et al., 2007):

- Transferral of the introduced pharma-specific sequences into sexually compatible neighboring food or feed crops followed by inadvertent eating
- Inadvertent admixing with food crops during harvesting and processing
- Inadvertent eating of the pharma plant could possibly lead to a toxic or allergic reaction
- Transferral of the introduced genes into microorganisms in the human intestines following inadvertent consumption of a GM plant
- Risk to workers

Great care should be taken that inadvertent consumption of pharma plants is avoided, because, if such plants are consumed especially by vulnerable groups (children, elderly, they might be harmed by the expressed protein. Transferral of pharma genes into microorganisms in the human gut is likely to be extremely rare, since DNA is broken down in the digestive tract, and thus in most cases only fragments of genes would be transferred as discussed in

Chapter 4.2. In principal, the risk that introduced sequences are transferred to other plants is the same as with food plants. A good summary of the steps in risk assessment of pharma plants can be found in the EFSA Guidance for the risk assessment of genetically modified plants used for non-food or non-feed purposes (EFSA, 2009).

The medicinal properties (benefits and risks) have to be assessed as for other medicinal products according to the regulations described above. Batch to batch consistency is a problem bigger with pharma plants than e.g. with plant or animal cell cultures:

Furthermore, standard production protocols (SPP) are also likely to form a fundamental part of the production of medicinal products which must achieve a high level of batch to batch consistency as a part of medicinal product regulation and approval. Though there are non-controllable variables under open field conditions (e.g. weather conditions, pathogens, soil quality), it may be useful and necessary to define in detail the methods of initial seed production and maintenance, the confinement measures, isolation distances, agronomic management, usage of fertilizer and segregation methods for the products from harvest to final market (EFSA, 2009).

Good manufacturing practices (GMP) are more difficult to adhere to with plants grown in open fields than with cell cultures. GMP has to be followed more strictly in Europe than in the US (Spök et al., 2008). In the consultation initiated by WHO in 2005³⁰, it was concluded that the GMP principles, as they are generally applied for production of pharmaceuticals, would have to be modified and supplemented when dealing with pharma crops and that process validation would be very difficult for open field cultivation.

Mitigation of risk

There are a number of ways to avoid the risk of transferral of pharma genes in pharma crops to other plants. In principle the measures for pharma crops are not different from those which can be applied to other crops. Mitigation of risk mainly concerns the environment, but also inadvertent mixing with food crops during cultivation in the field. Some of the measures are low-tech measures, but there are also a number of biological measures possible, discussed in more detail in (Alderborn et al., 2010; Daniell, 2002; Dunwell and Ford, 2005; Sparrow et al., 2007).

- Physical isolation of the crop
- Barrier crops
- Temporal isolation
- Physical removal of flowers
- Use of marker genes to make the crops or seeds visually distinctive from food or feed crops
- Introduction of a bitter or undesirable taste
- Addition of a transgenic silent identifier in PMPs and PIPs designed to enable technically simple identification
- Molecular containment such as plastid transformation, male sterility, terminator technology
- Engineered genetic containment such as apomixis, conditional lethality, inducible promoters, transgene mitigation.

General environmental risk issues including impact on soil and water will not be discussed here, since these risks are not inherently different from other GM plants grown in open field. This topic is discussed elsewhere (Bartsch and Sweet, 2012).

³⁰ <http://www.who.int/biologicals/Plant%20Vaccine%20Final%20Mtg%20Repor%20Jan.2005.pdf>

Conclusions

The most important advantages of PMPs compared to other protein expression systems (especially bacterial systems) are: the low risk of contamination with animal or human pathogens, relatively low investment and production costs, and good possibilities for scale-up, processing and modification of pharmaceutical proteins. PMPs have been limited to experimental and small-scale commercial production, yet industry's outlook now seems to be more optimistic, as judged from the fact that large pharmaceutical companies have invested in small PMP companies and there has been a push for large volume, more efficient production of vaccines in the US as well as in Europe. The feasibility and potential efficacy have well been established. It is possible that the first PMPs in use will be animal vaccines, because the demonstration of efficacy is easier in animals and the regulatory path is potentially shorter for them.

4. Risk Assessment

Any foreign DNA-construct introduced into a host plant results in an organism that is different from the wild type. Therefore, the resulting composition, the expected changes as well as unexpected changes should be investigated and compared to the isogenic control. *“An assessment of any potential for increased toxicity and/or allergenicity to humans and animals or for modified nutritional value due to the stacked events should be provided. These potential effects may arise from additive, synergistic or antagonistic effects of the gene products or by these produced metabolites and may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways. This assessment will clearly require a case-by-case approach.”* (EFSA, 2007). According to the EFSA guidance document the following approach is suggested:

- Risk assessment
- Comparative approach
- Concept of familiarity
- Concept of substantial equivalence
- Intended and unintended effects
- Environmental risk assessment (defined but refer to synthesis report by Bartsch and Sweet).
- Relevant issues and general recommendations/current knowledge

“Risk assessment” can be described as *“a process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)”* (EC 2000). A risk assessment comprises hazard identification, hazard characterization, exposure assessment, and risk characterization (Codex, 2001; EC, 2002).

The sequential steps in GMO risk assessment are: identification of characteristics that may cause adverse effects, evaluation of the potential consequences, assessment of the likelihood of occurrence and the estimation of the risks posed by the identified characteristics of the GMO (EC, 2002; EFSA, 2006, 2011).

For most of the GM plants it can be assumed that the host plants are traditionally cultivated crops with a history of safe use for the healthy consumer, animal and the environment. These host plants/isogenic lines should serve as a baseline/comparator for the safety assessment of GMOs (**concept of familiarity**) (OECD, 1993a, b; WHO/FAO, 2000). This concept of familiarity exploits the existing data set and knowledge on the well known and researched host crops and should facilitate the comparative safety assessment. This approach, also known as the **concept of substantial equivalence**, should identify similarities and potential differences between the GM-crop derived food and feed and the non-GM counterpart.

This analysis should include the molecular, chemical, agronomic and morphological characteristics of the target organism and the wild-type counterpart grown under the same agronomic conditions. The consecutive assessment includes specific safety and nutritional testing. All these data should provide evidence on whether or not the food /feed derived from GM-crops is as safe as the traditional counterpart and should identify both, **intended and unintended effects** (EFSA, 2006, 2011).

While intended effects are those that fulfill the original objectives of the genetic modification process, the unintended effects are defined as differences between the host plant and the GM plant beyond the primary expected effects.

The environmental risk of a GM-crop should also be evaluated and includes the hazard identification that summarizes the potential adverse effects on target and non-target biota, the effect on the trophic layer effects, affecting organisms not directly exposed and the exposure studies, the ecological risk assessment. For further information regarding ecological risk assessment see the accompanying Module 4 report by Bartsch and Sweet (2012). In addition to pre-market safety assessment, post-market surveillance approaches are discussed as valuable tools to evaluate long-term exposures of GM-derived food (Wal et al., 2003).

4.1 Methodological Aspects of Risk Assessment

In order to evaluate the safety of GM plants for food and feed according to the concept of substantial equivalence as described above, the characteristics of the GM food or feed are compared to existing food/feed crops. A number of methods have been developed in recent years. Originally, this consisted of a compositional analysis of the novel food compared to conventional food (Cellini et al., 2004). However, the system had to be refined, and it became obvious, that the comparisons could only be performed, if an exact baseline was determined, i.e. a GM crop line had to be compared to a parent line identical to the GM line apart from the inserted gene(s) (isogenic line) and that the crops had to be compared at different levels, i.e. at transcription level, protein expression etc. The comparative approach using baselines as reference points is also an important topic in the EFSA guidance document (EFSA, 2006, 2011). Figure 1 shows the different levels of analyses used to define substantial equivalence of GM crops. The figures show, from top to bottom, a Southern blot of plant DNA, part of a cDNA microarray, a two-dimensional gel of plant proteins and an NMR trace.

Genomics	Structure and organisation of genes
Transcriptomics	Expression of genes: level, location, stability
Proteomics	The complete spectrum of proteins present in an organelle, cell, tissue, or organism
Metabolomics	The entity of low molecular mass metabolites (sugars, amino acids, etc.)

Figure 1: Different levels of analysis for defining the substantial equivalence (modified from Shewry et al., 2007).

Shewry and coauthors compared GM and conventional wheat lines grown in greenhouses and in the field by using the above methodologies and, in addition, they compared the data from a number of studies on GM cereals. They came to the following conclusions for the transgenic wheat lines:

- 1. The expression of the transgenes in the lines studied is not intrinsically more or less stable than that of the corresponding endogenous genes.*
- 2. The transgenic and control lines show similar stability in agronomic performance and grain functional properties when grown at multiple sites and years.*
- 3. The gene expression profiles in developing grains of transgenic and control lines are much more similar to those of the parental lines than are the profiles of lines produced by conventional plant breeding.*

4. The metabolite profiles of control and transgenic lines usually fall within the range of variation which is observed between genotypes of the species or samples of the same genotype grown under varying environmental conditions (Shewry et al., 2007).

These data are supported by other studies: Batista et al. (Batista et al., 2008) analyzed gene expression in rice plants by oligonucleotide microarrays. The demonstrated that alteration (improvement) of a plant, either by conventional mutagenesis or by genetic modification, caused stress in the plant, leading to expression of untargeted genes. However, the alterations were more extensive in the mutagenized plants than in the genetically modified ones. Similar results can also be found elsewhere (Baker et al., 2006; Flachowsky et al., 2007). Recent publications using the so-called “*omics*” technologies have demonstrated that differences between plants in most cases depended on the area of cultivation, fertilization treatment, climate, and the variety of the plant (cultivar). This was shown among others with barley (Coll et al., 2010; Kogel et al., 2010), and maize, in this specific case the commercially cultivated MON810 maize (Coll et al., 2010). Extensive reviews of the “*omics*” technologies for food safety assessment have been published (Chassy, 2010; Davies, 2010a).

4.2 Risk Assessment Recommendations

Risk assessment of GM plants should aim at identifying any **adverse or toxic** effect. Suitable tests are based on standardized toxicological methodology and designed for the assessment of defined chemical substances. Standard guidelines exist on how to conduct such tests under good laboratory practice (GLP) conditions (OECD Guidelines for Testing of Chemicals (EFSA, 2008; OECD, 1995). Nevertheless, testing single potential toxic substances is different to testing whole food and feed products with a complex composition.

The studies necessary to investigate potential toxicity of a newly expressed protein should be performed on a case-by-case basis (see above). Detailed information on the description of the newly inserted gene and the resulting protein, the DNA-construct and the transgene delivery method is necessary (EFSA, 2008). Further key elements of this assessment are the compositional analysis of the GM plant and of the derived food or feed product. For evaluating the safety and nutritional performance of the GM product animal feeding trials should be performed (EFSA, 2008). In the recent past, integrated efforts to investigate the health food aspect together with an environmental safety approach were discussed (Haslberger, 2006).

Animal studies

In order to address a potential health hazard due to consumption of GM-derived food animal studies are the method of choice. These studies should provide evidence whether GM-food intake poses an increased health risk or not. Some feeding studies are designed as models for risk assessment for both, humans and animals, and they use **laboratory animal models** (e.g. mice, rats). Other studies use **target animals**, mostly cattle, sheep, poultry, and pig to study the effect of GM-feed on the health of food producing animals. Depending on the chosen animal model, individual parameters of health versus sickness have to be taken into account. While highly toxic reactions can be detected rather early during a feeding trial, long-term effects will only be detected in trials with an adequately long time frame applied. Questions related to fertility can be addressed in multi-generation studies, and are only feasible for certain animal models. Recently, the so-called BEETLE report, issued by the German Federal Office of Consumer Protection and Food Safety summarized the current knowledge on long-term effects of GM-crops on health, biodiversity and environment. This report provides an up to date review of health-related aspects for animals and humans (BEETLE, 2009).

Risk assessment of toxicity

*“The risk assessment of GM plants and derived food and feed follows a comparative approach, i.e. the derived food and feed are compared with their non-GM near isogenic counterparts in order to identify differences which subsequently are assessed with respect to their potential impact on the environment, safety for humans and animals and nutritional quality (**Concept of Substantial Equivalence or Comparative Safety Assessment**). This approach has been developed and accepted by international organisations like the EC, the FAO/WHO Codex Alimentarius and OECD.” (EFSA, 2008; Panel, 2008).*

The **comparative risk assessment** approach for GM plant-derived food and feed is a stepwise procedure and considers safety issues related to the intrinsic properties of the introduced foreign gene and the issues related to unintended effects, such as overall impact on the plant genome. It is generally agreed that such a risk assessment is done on a case by case basis and should include data on the molecular, compositional phenotypic, agronomic nature of the GM product in comparison with the natural counterpart. The required analyses include **in silico, in vitro and in vivo methods**. Results of the in silico and in vitro methods should help to decide whether in vivo animal studies are required (see Fig. 2).

The following background information on the GM plant is necessary:

- The parent plant (history of safe use in the past, phenotype, chemical composition)
- The type of transformation process (source of transgene, DNA construct, information on possible DNA insertion)
- Heterologous expressed protein(s) and other substances (toxicity or allergenicity)
- GM plant (agronomic and phenotypic traits, composition, safety and nutritional characteristics, ability to outcross or transfer genetic material to other organisms)
- Anticipated intake/extent of use, exposure?
- Nutritional properties
- Storage and food processing conditions

The current performance of the safety assessment of whole foods derives from the **toxicology risk** assessment, originally designed for low-molecular weight chemicals. Therefore, these animal tests should be adapted when testing whole food and feed (complex mixtures of compounds with different biological characteristics). Apart from many other animal tests, the 90-day rodent animal feeding trial has been commonly used and has the capacity to detect potential toxicological effects for single substances. In such feeding trials a number of parameters are recorded such as body weight, feed consumption, blood chemistry, organ weights, histopathology etc. Special attention has to be paid to design the animal diet according to the known nutritional requirements in order to omit any malnutrition. While 90 day feeding studies have the potential to identify potential toxicological effects, low levels of unintended effects will not be recorded by that animal test. Adverse effects on reproduction cannot be identified either and require other studies such as multi-generation studies (see also “Animal studies” page 29).

For further details related to animal tests for the safety assessment of GM plants see EFSA Report 2008 (EFSA, 2008).

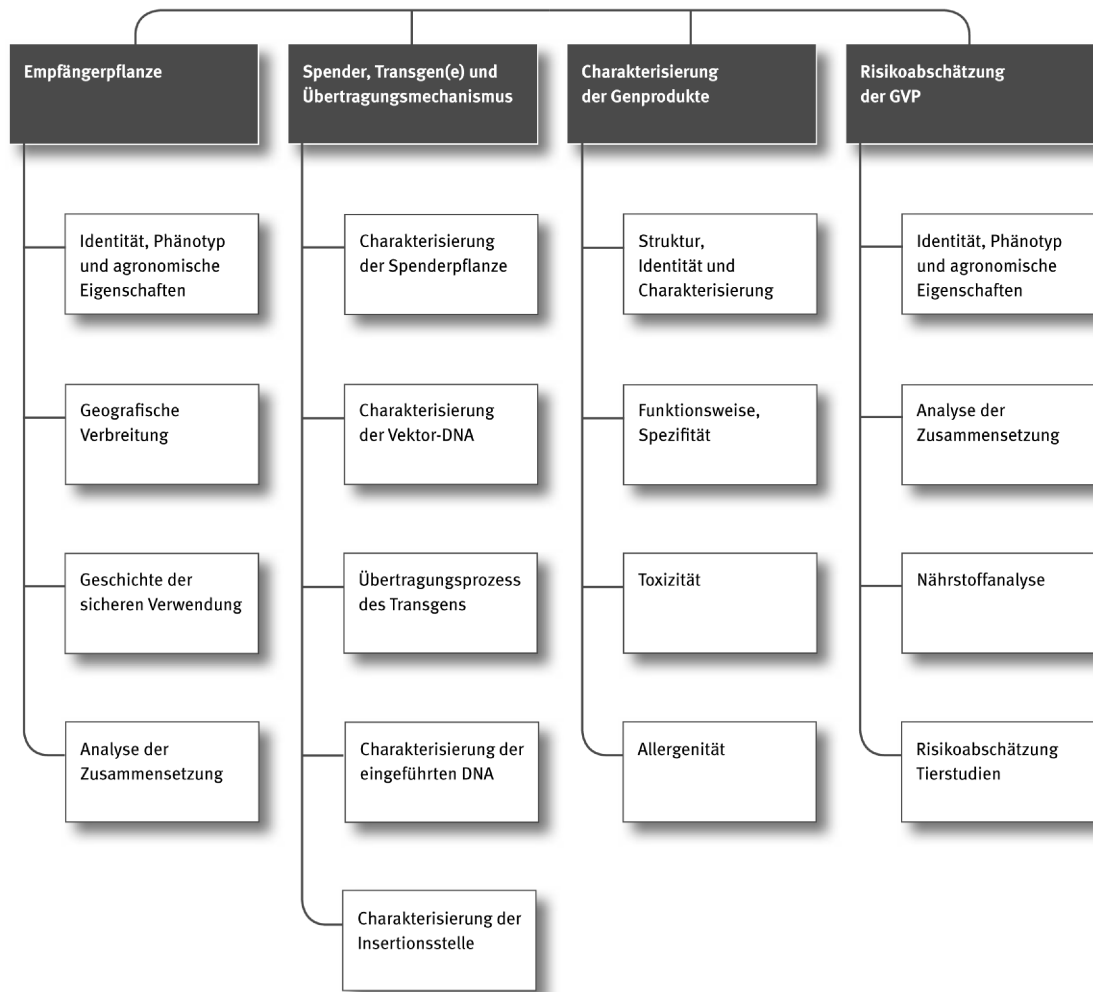


Figure 2: Integrative approach for hazard assessment (modified from EFSA, 2008).

In the recent report by the EFSA (EFSA 2010) panel on the safety assessment of GM plants and derived food and feed it was stated that the integration of new profiling technologies like **transcriptomics, proteomics and metabolomics** into the experimental risk assessment is appreciated and will facilitate also the detection of unintended effects in a non-targeted approach. However, these new methods will need validation before routine use. In addition, it became evident that there is a need for a more uniform approach regarding the design and especially the **statistical analysis** of animal feeding trials. The discrimination of observed differences either due to potential GM-related hazardous effects or based on normal individual biological variation is a crucial element in this context. **Post-market monitoring** cannot be regarded as a standard requirement and cannot replace pre-market risk assessment. However, it may give additional information regarding consumer habits, frequency of exposure to previously unknown substances and could help to identify potential risks in certain cases (e.g. immunological disorders).

Last but not least, it should be noted that most of the recommendations are based on the safety investigations on herbicide and insect resistant GM plants. Whether these regulations are also sufficient for the whole range of 2nd and 3rd generation GM plants remains to be re-evaluated.

As stated above, the **allergenic risk assessment of GM plants** differs from the toxicological risk assessment, since it affects predisposed individuals and no general threshold doses are

given for individual food allergens. In 1996, the International Food Biotechnology Council (IFBC Washington, DC) together with the International Life Science Institute (ILSI) Washington, DC) have formulated the first guidelines for evaluating the allergenicity of GMOs. In 2001 the WHO published guidelines together with the FAO, and in 2003 the Codex Alimentarius formulated their guidelines (Codex, 2003). EFSA has published guidelines in 2004 (EFSA, 2004) which were adapted in 2010 (EFSA, 2010) .

Although the WHO/FAO guidelines differ from the ones from EFSA, they share the opinion that allergenic risk assessment should include in silico analysis of the target sequence, pepsin digestion assays of the target protein and serum testing using allergic patients sera. While FAO/WHO recommend following a **decision tree** for allergenic risk assessment, Codex Alimentarius and EFSA (see Table 7) define the risk assessment as a **weight of evidence** approach.

Table 7: Recommendations for allergenic risk assessment of GM plants

Recommendations	
FAO/WHO	Codex Alimentarius Commission, EFSA
“Decision Tree”	“Weight of evidence approach”
Sequence analysis	Sequence analysis
Target Serum IgE tests, skin prick tests	Target Serum IgE tests
Pepsin digestion assay	Pepsin digestion assay
Animal model	

Although threshold values of 35% for sequence similarity (over a window of 80 amino acid residues) and above to a known allergen need further testing, FAO/WHO take 6 and IFBC/ILSI 8 continuous amino acid residues identical to a know allergen as a threshold for further clinical serum testing. In the recent past, the 6 or 8 amino acid rule has been criticized as not sensitive enough, giving rise to too many unspecific positive hits. By this method, about 40% of proteins from the human genome can be considered as allergenic (Stadler and Stadler, 2003). However, the sequence similarity threshold is still a matter of debate and new, more refined algorithms are awaited to contribute to a more precise analysis. An example of a more refined method would be the search for protein motifs relevant for allergens as Stadler and Stadler have developed (Stadler and Stadler, 2003). Furthermore, stability of a protein should be assessed in an in vitro digestion assay. The underlying assumption is that a food allergen displays more allergenic activity the longer it remains as an intact protein in the digestive tract. However, this stability feature does not account for all allergens, and examples of food allergens with low stability are also reported.

Serum testing with sera from patients with a diagnosed allergy to the target protein and/or the wild-type organism is recommended. In addition, Skin Prick Tests and Double Blind Placebo Controlled Food Challenges are recommended by the IFBC/ILSI in the case of a positive hit with the sequence of a known allergen. In contrast, WHO/FAO do not recommend these in vivo tests for ethical reasons. Animal tests for evaluating a potential increased allergenic risk of a GM-derived protein are recommended by EFSA if there is evidence for potential allergenicity (EFSA, 2006). However, it has to be stated that at present no

commonly accepted animal model for allergenic risk assessment is available. Whether positive immunization of animals in an experimental setting with subsequent allergic reactivity to the target protein is a true indication of an increased allergenic risk remains to be investigated in more detail.

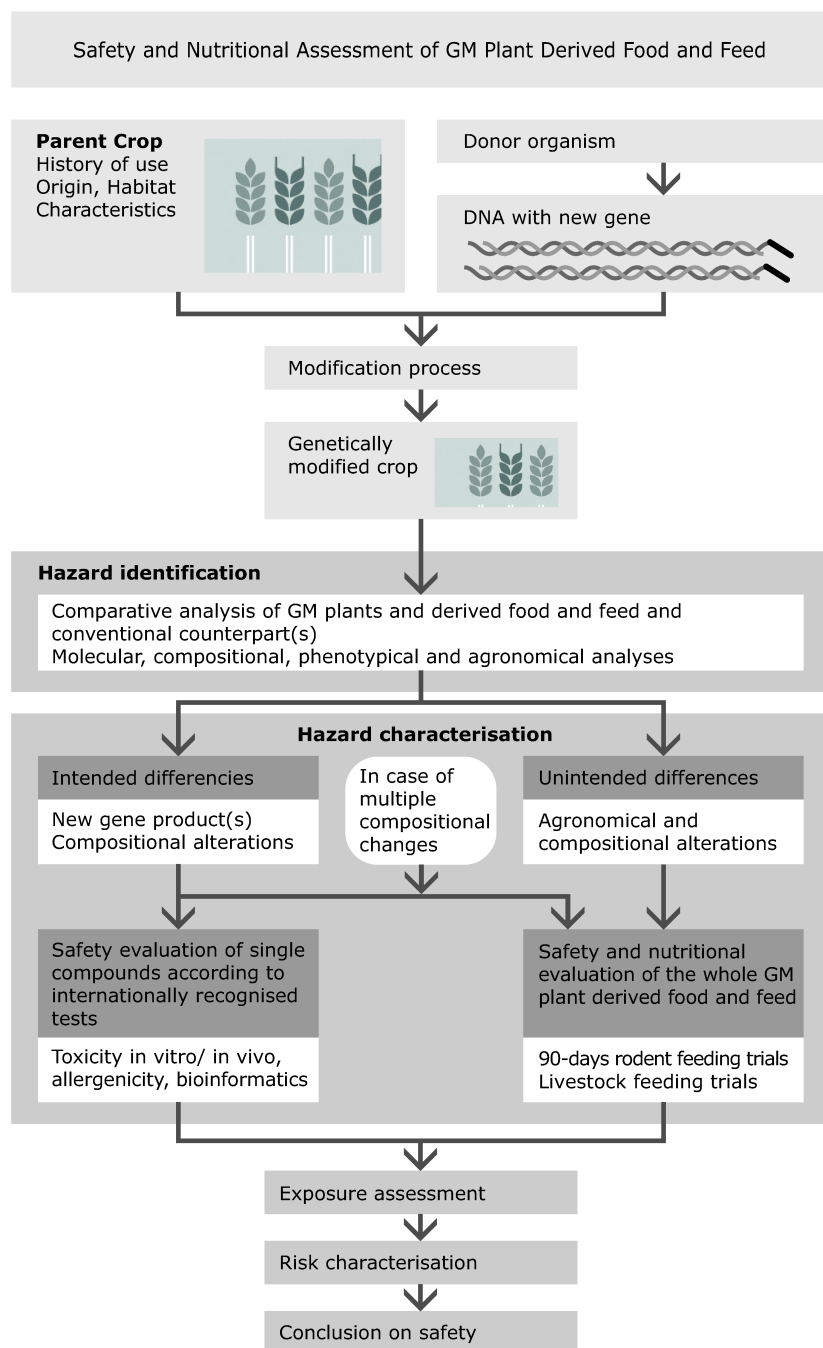


Figure 3: Strategy for safety and nutritional testing of GM plant derived food and feed based on EFSA recommendations (EFSA, 2008).

In summary, there is an awareness of a potential allergenic risk due to a GM plant and it is clear that certain analyses should help to minimize the risk. In principle, these risk analyses are based on the knowledge about already known allergens; the possibility of allergenic activity of an up to now unknown protein is limited.

In silico analyses and serum testing from well-defined patients contribute to minimize the risk. The weight of evidence approach provided by entire data set is still considered to give a sufficient degree of safety for the heterologously expressed proteins of primarily microbial origin from the 1st generation of GM plants. However, the challenge is, whether this type of risk assessment is still applicable for 2nd and 3rd generation of GM plants? Therefore, new state of the art biophysical characterization methods and in silico methods as well as future efforts for the identification of validated animal tests should be included in improving the current allergenic risk assessment for GM plants.

5. Conclusions

In parallel with the construction of 1st generation GM plants, methods related to risk perception and risk management of GMOs have been developed and the number of studies investigating potential effects on human and animal health has increased steadily over the last 2 decades. Guidelines were formulated by international authorities and have been more or less regularly updated since then. In contrast, no such detailed regulations are set up for plants mutated either chemically or by radiation, especially food crops and novel foods.

The 1st generation of GM plants was primarily devoted to increase insect- and herbicide-resistance (i.e. Bt-toxins and glyphosate and glufosinate-tolerance) and to improve agronomic performance, which is also reflected by the respective large number of risk assessment studies.

Gaps: In the 2nd and 3rd generation of GM plants the focus has changed and the spectrum of possible changes due to the expression of GM products has broadened. The plethora of GM products implies that also the risk assessment needs to be adapted accordingly. We are no longer dealing with 2-3 constructs; there is now a whole range of new target sequences, up- and down-regulation of either beneficial or adverse substances and different organelles serving as heterologous expression systems. The GM-product can either improve agronomic performance and/or harvest and storage conditions, but also features related to nutritional value can be modified. Finally the use of GM plants to produce pharmaceutical compounds has offered new possibilities. All these aspects need to be addressed to formulate and adopt methods of risk assessment previously not anticipated. Regulations for GM plant derived pharmaceuticals have to be adopted accordingly.

Guidelines on how to perform risk assessment of GM plants were set up by international authorities (WHO/FAO, Codex Alimentarius, EFSA, EMEA) and the respective legislation (EU, national level) has been set up for 1st generation GM-plants. It is generally accepted – even if the recommendations differ among the authorities – that studies have to be performed to assess intended and unintended effects of a GM plant. These tests should be performed according to generally accepted protocols, based on the **concept of familiarity and substantial equivalence** on a case-by-case basis.

Gaps: At least for allergenicity assessment a weight of evidence approach should be applied. These recommendations help to identify already known allergens and their homologous sequences but the methods are not efficient in assessing yet unidentified allergens.

Production of medicinal products from PMPs should be done under **Good Manufacturing Practice (GMP) conditions**. However, it can be difficult to apply GMP principles to GM plant cultivation, harvest and primary processing of harvested material. The EMEA guideline which came into effect in 2009³¹ states *“Where classical GMP principles prove impractical to apply to elements of this phase, a suitable Quality System should be developed and put in place.”* It is evident that the legislation for PMPs - and in part for nutraceuticals - is only evolving now. Production processes under GMP conditions and risk assessment will have to be defined in more detail, since the first products are now in clinical phase trials and clear regulations are necessary.

General Recommendations and Gaps:

For the overall risk assessment of GM plants it is mandatory to continuously adapt regulations and recommendations: Science evolves and new risks have to be assessed, but

³¹ http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003154.pdf

on the other hand new technologies are available to fine-tune the risk assessment, especially for 2nd and 3rd generation of GM plants.

Toxic risk assessment is based on tests identifying rather severe health problems, whereas mild long-term effects are difficult to detect and appropriate tests are lacking or insufficient. A number of animal models are used in these risk assessment studies and all these models have their limitations. So, far no validated animal model is available. In addition, a number of different statistical methods have been used in the risk assessment in the past resulting in sometimes contradicting results. Authorities have recognized that there is a need to harmonize methods and their application for risk assessment, and work to solve this problem is ongoing.

Post-market monitoring of GM plants and products thereof has been under debate for a long time. The methods for post-market monitoring are still being developed. It is generally recognized that it has to be done on a case-by-case basis and that it should rather be applied as an instrument for recording information in case of unintended effects.

In the last 2 decades numerous studies were performed to assess any potential health hazard of GM food and feed in animals or humans. While the majority did not detect adverse effects, there are reports providing data on the contrary (Seralini et al., 2007). However, these studies were reviewed by EFSA panels and other researchers on a case-by-case approach, and so far no adverse effects have been confirmed.³² A detailed summary can be found in the BEETLE-Study (BEETLE, 2009).

Pharma plants could be a good option for research in applied science and for industrial production in Switzerland, especially since Switzerland has a long history in pharmaceutical research and industry. Syngenta is at the moment not doing any research with food or pharma plants in Switzerland (P. Ahl-Goy, Syngenta, personal communication), one reason being that the difficulties with field studies are too big in Switzerland. But there are other options with PMPs that can be exploited in Switzerland: plant cultures in green houses or plant cell cultures do not require field trials. However, unless only preclinical research is being done, GMP facilities for production as well as setting up regulations for dealing with pharma plants in Switzerland would be required. Research on transgenic plants is at a high level in Switzerland and promotion of this type of research should not stop including field trials for scientific purpose if required while taking all necessary precautions into account.

Another research area, where relevant Swiss expertise is available comprises various areas of risk assessment of GMOs. However, whether this expertise can be further developed and stay competitive in the international scientific community depends on the facilities and on options offered to the scientific community and needs to be discussed by decision makers and the public (Nordlee et al., 1996) (Oberdorster, 2001).

³² <http://www.efsa.europa.eu/>

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Additional relevant links:

Agbios	http://www.agbios.com/main.php
Biosicherheit Germany	http://www.biosicherheit.de/de/
Center for Environmental Risk Assessment	http://cera-gmc.org/
GMO Compass	http://www.gmo-compass.org/eng/home/
(English version of Biosicherheit.de)	
Dialog Gentechnik Austria	http://www.dialog-gentechnik.at/intro.html
EFSA	http://www.efsa.europa.eu/
EU EUR-lex	http://eur-lex.europa.eu/en/index.htm
GMO info	http://gmoinfo.jrc.ec.europa.eu/
Internutrition (CH)	http://www.internutrition.ch/index.html
Pharma-Planta	http://www.pharma-planta.org/

7. Abbreviations

Bt	<i>Bacillus thurigiensis</i>
CHO	Chinese Hamster Ovary
DNA	Deoxyribonucleic acid
DsRNAi	Double-stranded RNA interference
EFSA	European Food Safety Authority
EMA	European Medicines Agency
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (Miles et al.)
GLP	Good Laboratory Practice
GM plants	genetically modified plants
GMP	Good Manufacturing Practice
GMO	genetically modified organism
ILSI	International Life Science Institute
JRC	Joint Research Center of the European Commission
OECD	Organisation for Economic Co-operation and Development
PMI	plant made industrials
PMP	plant made pharmaceuticals, pharma plants
TA	technology assessment
USDA	United States Department of Agriculture
WHO	World Health Organization

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9. Appendix: EU-Regulations

Food and feed regulations

Regulation (EC) No 298/2008 of the European Parliament and of the council of 11 March 2008 amending Regulation (EC) No 1829/2003 on genetically modified food and feed, as regards the implementing powers conferred on the Commission.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:097:0064:0066:EN:PDF>

Commission Regulation (EC) No 65/2004 of 14 January 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:010:0005:0010:EN:PDF>

Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0024:0028:EN:PDF>

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0001:0023:EN:PDF>

Regulation (EC) No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on transboundary movements of genetically modified organisms (Applies for export of GMOs to third countries and for transboundary movements between Member States).

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:287:0001:0010:EN:PDF>

Council Directive 2002/53/EC of 13 June 2002 on the common catalogue of varieties of agricultural plant species (Regulations on GM seeds and other plant-propagating material).

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:193:0001:0011:EN:PDF>

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority (EFSA) and laying down procedures in matters of food safety.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:031:0001:0024:EN:PDF>

Directive 2001/18/EC of the European Parliament and of the council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:106:0001:0038:EN:PDF>

Council Directive 98/81/EC of 26 October 1998 amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:330:0013:0031:EN:PDF>

Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market (Risk assessment of plant protection products used directly in the cultivation of crop plants).

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1991:230:0001:0032:EN:PDF>

Regulation of pharma plants

Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorization and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:136:0001:0033:EN:PDF>

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. (Applies to plant products to be used for human or animal consumption).
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0001:0023:EN:PDF>

Council Directive 98/81/EC of 26 October 1998, amending Council Directive 90/219/EEC of 23 April 1990 on the **contained use** of genetically modified micro-organisms.
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:330:0013:0031:EN:PDF>

This literature review is part of the National Research Programme NRP 59
“Benefits and Risks of the Deliberate Release of Genetically Modified Plants”.
It is one of a total of three such reviews, which are:

- Medical issues related to genetically modified plants of relevance to Switzerland
- Genetically modified crop production: social sciences, agricultural economics, and costs and benefits of coexistence
- Synthesis and overview studies to evaluate existing research and knowledge on biological issues on GM plants of relevance to Swiss environments

They aim to distil relevant scientific data from the results of international studies on GMP that could help to shape future research and decision-making processes in Switzerland.



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