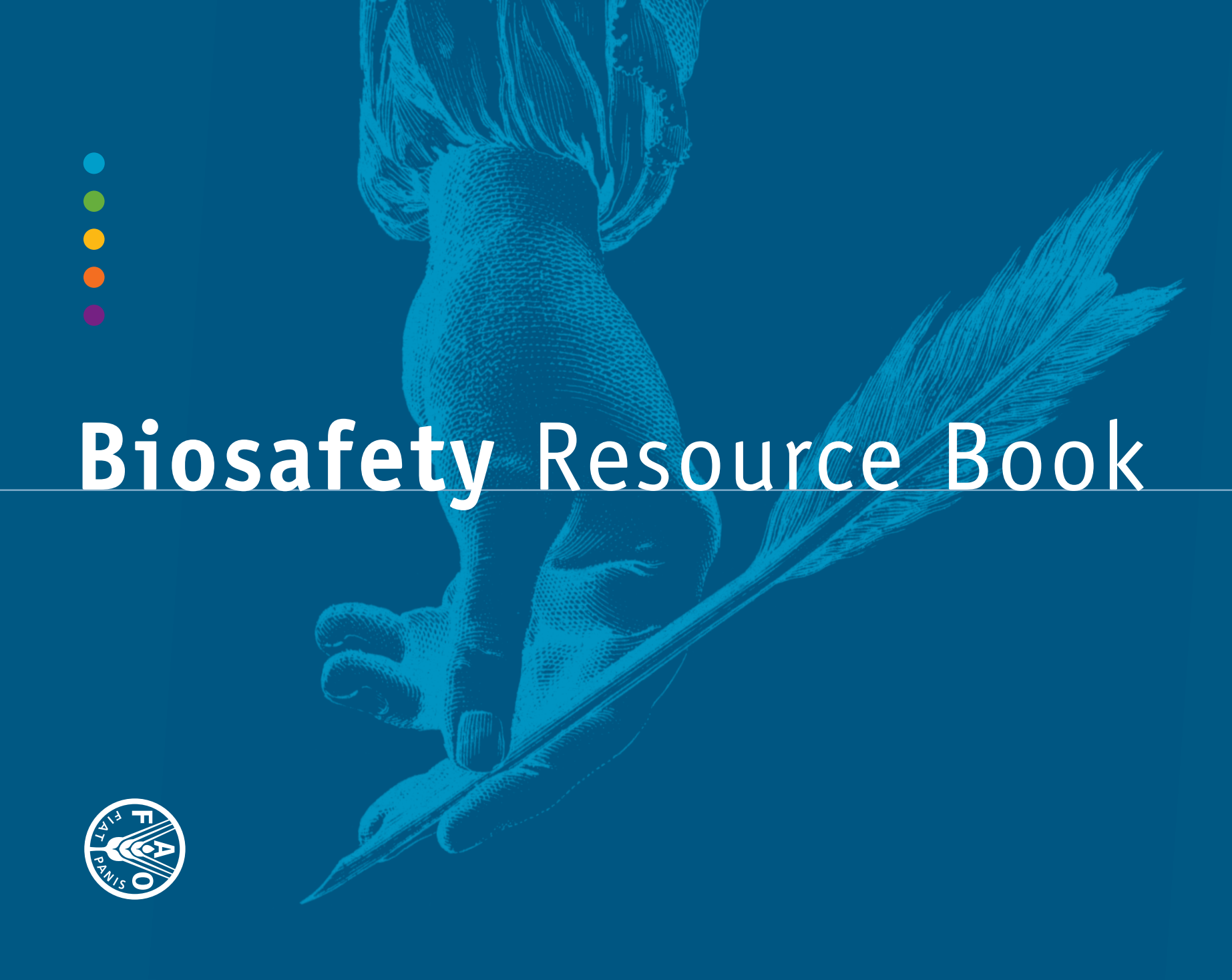


Biosafety Resource Book

The training programme consisted of the following modules:

MODULE a **MOLECULAR BIOLOGY AND GENETIC ENGINEERING**, which reviews the very basic scientific concepts and principles employed in producing GMOs, and provides a brief description of current and emerging uses of biotechnology in crops, livestock and fisheries.

MODULE b **ECOLOGICAL ASPECTS**, which provides the necessary background information on ecology and evolution needed to analyse and understand the consequences of introducing GMOs into the environment.

MODULE c **RISK ANALYSIS**, which provides basic information on biological risks, concepts, principles, and methodologies of risk assessment, risk management and risk communication. It focuses on crop biotechnology and environmental risk assessment of GM crops since these are of immediate interest to most countries.

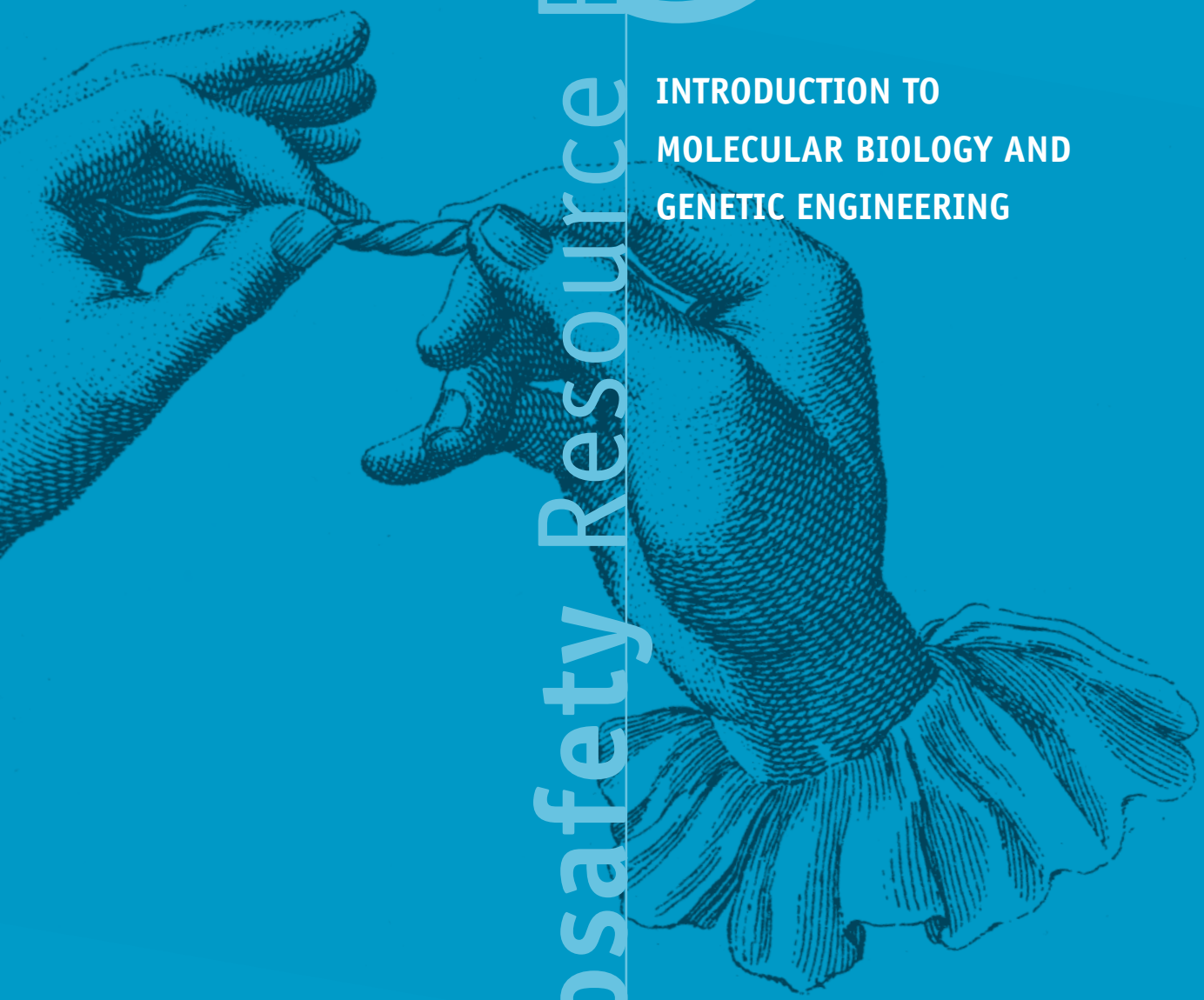
MODULE d **TEST AND POST-RELEASE MONITORING OF GMOs**, which addresses the use and monitoring of GMOs under containment, confinement and limited field trials, as well as the monitoring of commercially released GMOs. It also covers surveillance and emergency planning.

MODULE e **LEGAL ASPECTS**, which provides an overview of the existing legal tools and frameworks on biotechnology and biosafety, and offers a thorough description of the international instruments that regulate biosafety and their interactions.

Biosafety Resource Book

MODULE a

INTRODUCTION TO MOLECULAR BIOLOGY AND GENETIC ENGINEERING



FIAT PANIS

MODULE

Biosafety Resource Book

INTRODUCTION TO MOLECULAR BIOLOGY AND GENETIC ENGINEERING

Oliver Brandenberg
Zephaniah Dhlamini
Alessandra Sensi
Kakoli Ghosh
Andrea Sonnino

Food and Agriculture
Organization of
the United Nations
Rome, 2011

FOREWORD

During the period 2002–10, FAO undertook an intense activity of biosafety capacity development, largely centred on enhancing the capacities of regulators and other technical staff involved in the implementation of biosafety frameworks, along with other components. The training programme was tailored to meet the needs of a very specific audience: biosafety regulators, policy-makers and members of national biosafety committees, with diverse educational backgrounds, not necessarily well versed in all the biosafety-related fields. The training courses therefore aimed to: i) offer background knowledge critical in the process of reviewing biosafety dossiers and biosafety-related decision-making; ii) provide acquaintance with concepts and methodologies relevant to risk analysis of GMO release and biosafety management.

The training programme consisted of the following modules:

MODULE A

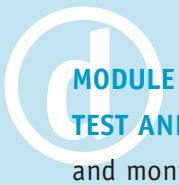
MOLECULAR BIOLOGY AND GENETIC ENGINEERING, which reviews the very basic scientific concepts and principles employed in producing GMOs, and provides a brief description of current and emerging uses of biotechnology in crops, livestock and fisheries.

MODULE B

ECOLOGICAL ASPECTS, which provides the necessary background information on ecology and evolution needed to analyse and understand the consequences of introducing GMOs into the environment.

MODULE C

RISK ANALYSIS, which provides basic information on biological risks, concepts, principles, and methodologies of risk assessment, risk management and risk communication. It focuses on crop biotechnology and environmental risk assessment of GM crops since these are of immediate interest to most countries.



MODULE D

TEST AND POST-RELEASE MONITORING OF GMOs, which addresses the use and monitoring of GMOs under containment, confinement and limited field trials, as well as the monitoring of commercially released GMOs. It also covers surveillance and emergency planning.

MODULE E

LEGAL ASPECTS, which provides an overview of the existing legal tools and frameworks on biotechnology and biosafety, and offers a thorough description of the international instruments that regulate biosafety and their interactions.

FOREWORD

This Biosafety Resource Book stems from experience gained in biosafety capacity development projects and is based on the materials developed by the lecturers who have taught in the training courses organized to date. The Resource Book has been prepared in response to an expressed need, with the purpose of being used as a training tool in future activities. The Resource Book also aims at providing biosafety regulators, policy-makers and members of national biosafety committees with reference materials that can be readily consulted beyond the training events, when the need arises. Special attention has been paid to avoid technical jargon and to keep the modules scientifically accurate as well as accessible to non-specialists.

FAO's biosafety capacity building activities are the result of a collaborative effort, involving numerous institutions, including national biosafety committees of many countries, ministries, universities and research institutes, NGOs and the private sector. The precious contribution of national project coordinators, national and international consultants, as well as FAO officers from headquarters and decentralized offices, is gratefully acknowledged. The enthusiasm and dedication of the participants in the training activities were crucial for their success.

Andrea Sonnino
Chief,
Research and Extension Branch
Office of Knowledge Exchange,
Research and Extension

LIST OF CONTRIBUTORS

This module has been prepared based on the lectures and contributions of:

Oliver Brandenberg
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
Institute of Medical Virology,
University of Zürich,
Zürich, Switzerland)

Zephaniah Dhlamini
Department of Applied Biology & Biochemistry
National University of Science and Technology
Ascot, Bulawayo, Zimbabwe

Richard Edema
Department of Biotechnology
Makerere University
Kampala, Uganda

Hrvoje Fulgosi
Department of Molecular Biology
Rudjer Boskovic Institute
Zagreb, Croatia

Kakoli Ghosh
Plant Protection and Production Division
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

Antonietta Gutiérrez-Rosati
Universidad Nacional Agraria
La Molina, Lima, Perú

Andrew Kiggundu
National Agricultural Biotechnology Laboratory
Kawanda, Uganda

Jesse Machuka
Department of Biochemistry and Biotechnology
Kenyatta University
Nairobi, Kenya

Hosea Ken Matengo
Department of Molecular Biology and
Biotechnology
University of Dar-es-Salaam
Dar es Salaam, United Republic of Tanzania

Emmarold E. Mneney
Mikocheni Agricultural Research Institute (MARI)
Dar es Salaam, United Republic of Tanzania

Sam Kwame Offei
Department of Crop Science
University of Ghana
Legon, Ghana

Leonard O'Garro
Department of Biological and Chemical Sciences
Faculty of Pure and Applied Sciences
University of West Indies
Cave Hill, Barbados

Ajaj Parida
M. S. Swaminathan Research Foundation (MSSRF)
Chennai, India

Alessandra Sensi
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
EuropeAid Co-operation Office, Unit A.3
Centralised Operations for Europe, the
Mediterranean and the Middle East
Brussels, Belgium)

Andrea Sonnino
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

CONTENTS

MODULE a

LIST OF ABBREVIATIONS

A	Adenine
AI	Artificial insemination
ALS	Acetolactate synthase
Amp	Ampicillin
ARS	Autonomously replicating sequence
ATP	Adenosine triphosphate
A. tumefaciens	*Agrobacterium tumefaciens*
BAC	Bacterial artificial chromosome
bp	base pair
BSE	Bovine Spongiform Encephalopathy
Bt	*Bacillus thuringiensis*
C	Cytosine
CaMV	Cauliflower mosaic virus
CAT	Chloramphenicol acetyltransferase
CBD	Convention on Biological Diversity
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
E. coli	*Escherichia coli*
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ES cell	Embryonic stem cell
ET	Embryo transfer
FAO	Food and Agriculture Organization of the United Nations
FDA	U.S. Food and Drug Administration
G	Guanine
GFP	Green fluorescent protein
GMO	Genetically modified organism
GUS	Beta-glucuronidase
HPLC	High performance liquid chromatography
ISO	International Organization for Standardization
ISTA	International Seed Testing Agency
IVF	*In vitro* fertilization
kb	kilobase
MAS	Marker-assisted selection
Mb	Megabase
MCS	Multiple cloning site
mRNA	Messenger RNA
OPU	Oocyte pick-up
ORF	Open reading frame
ori	Origin of replication
PAT	Phosphinothricin acetyltransferase
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PPT	Phosphinothricin
QTL	Quantitative trait loci
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
RT-PCR	Reverse transcriptase PCR
S. aureus	*Staphylococcus aureus*
SCNT	Somatic cell nuclear transfer
SMG	Selectable marker gene
snRNP	small nuclear ribonucleoproteins
ST	Somatotropin
T	Thymine
T-DNA	Transfer DNA
Ti	Tumour-inducing
TMV	Tobacco mosaic virus
tRNA	Transfer RNA
U	Uracil
UTR	Untranslated region
vir genes	virulence genes
YAC	Yeast artificial chromosome

For further explanation of terminology and other abbreviations, please refer to the FAO "Glossary of biotechnology for food and agriculture" (FAO, 2007), which is also available online at: http://www.fao.org/biotech/index_glossary.asp?lang=en

CHAPTER 1

INTRODUCTION TO BIOTECHNOLOGY: BASIC CONCEPTS AND DEFINITIONS

1.1 DEFINITION OF BIOTECHNOLOGY

The term biotechnology was coined in 1919 by Karl Ereky, a Hungarian engineer. At that time, the term included all the processes by which products are obtained from raw materials with the aid of living organisms. Ereky envisioned a biochemical age similar to the stone and iron ages.

Nowadays, according to the Convention on Biological Diversity (CBD), **Biotechnology** is defined as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (CBD, 1992). The living organisms or derivatives thereof most frequently used include micro-organisms, animals and plants (or their isolated cells) as well as enzymes. They can be utilized to process substances, usually other natural, renewable materials, or serve themselves as sources for valuable substances or goods. Several branches of industry rely on biotechnological tools for the production of food, beverages, pharmaceuticals and biomedicals. The CBD definition is applicable to both "traditional" or "old" and "new" or "modern" biotechnology.

BIOTECHNOLOGY
Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Long before the term biotechnology was coined for the process of using living organisms to produce improved commodities, people were utilizing living micro-organisms to obtain valuable products, for example through the process of fermentation.

A list of early biotechnological applications is given below in Table 1.1:

Table 1.1 | **Traditional applications of biotechnology**

Providing bread with leaven	Prehistoric period
Fermentation of juices to alcoholic beverages	Prehistoric period
Knowledge of vinegar formation from fermented juices	Prehistoric period
Manufacture of beer in Babylonia and Egypt	3rd century BC
Wine manufacturing in the Roman Empire	3rd century AD
Production of spirits of wine (ethanol)	1150
Vinegar manufacturing industry	14th century AD
Discovery of the fermentation properties of yeast	1818
Description of the lactic acid fermentation by Pasteur	1857
Detection of fermentation enzymes in yeast by Buchner	1897
Discovery of penicillin by Fleming	1928
Discovery of many other antibiotics	≈1945

Since the middle of the twentieth century biotechnology has rapidly progressed and expanded. In the mid-1940s, scale-up and commercial production of antibiotics such as penicillin occurred.

The techniques used for this development were:

- isolation of an organism producing the chemical of interest using screening/ selection procedures, and
- improvement of production yields via mutagenesis of the organism or optimization of media and fermentation conditions. This type of biotechnology is limited to

chemicals occurring in nature. It is also limited by its trial-and-error approach, and requires a lengthy procedure over years or even decades to improve yields (Rolinson, 1998).

About three decades ago, with the advance of molecular biology, biotechnology became more of a science than an art. Regions of deoxyribonucleic acid (**DNA)** (called genes) were found to contain information that directs the synthesis of specific proteins. Proteins can therefore be considered as the final product of a gene; they are the molecules that carry out almost all essential processes within a cell. Each protein has its own identity and function: many are so-called enzymes that catalyse (facilitate) chemical reactions, others are structural components of cells and organs (Morange and Cobb, 2000). Today it is possible to express a gene, regardless of its origin, in a simple bacterium such as *Escherichia coli (E. coli)*, so that the bacterium produces large quantities of the protein coded for by the gene. The same principle can be applied to many other micro-organisms, as well as to higher organisms such as plants and animals.

DNA
Acronym for Deoxyribonucleic Acid: The material in which our hereditary information is stored.

The techniques used for this purpose include:

- isolation of the gene coding for a protein of interest;
- cloning (i.e. transfer) of this gene into an appropriate production host;
- improving gene and protein expression by using stronger promoters, improving fermentation conditions etc. (Gellisen, 2005). Together, these techniques are known as **recombinant DNA technology** and will be discussed at some length throughout this resource book.

RECOMBINANT DNA TECHNOLOGY
The process of constructing and manipulating DNA sequences that do not occur naturally, by combining DNA fragments from different sources.

About two decades ago, protein engineering became possible as an offshoot of the recombinant DNA technology. Protein engineering differs from "classical" biotechnology in that it is concerned with producing new (engineered) proteins which have been modified or improved in some of their characteristics (Park and Cochran, 2009).

The techniques involved in protein engineering are essentially based on recombinant DNA technology and involve:

- various types of mutagenesis (to cause changes in specific locations or regions of a gene to produce a new gene product);
- expression of the altered gene to form a stable protein;
- characterization of the structure and function of the protein produced;
- selection of new gene locations or regions to modify for further improvement as a result of this characterization.

The commercial implications of the technical developments listed above are that a large number of proteins, existing only in tiny quantities in nature, can now be produced on an industrial scale. Furthermore, the yields of biochemical production can be increased much faster than what was originally possible with classical fermentation.

GENETICALLY MODIFIED ORGANISM
An organism whose genetic material has been modified, for example by introducing foreign DNA sequences obtained by recombinant DNA technology.

Importantly, the production of transgenic animals and plants that contain genetic elements from foreign sources and possess novel traits and characteristics is also based on the techniques outlined above. As all these approaches result in the creation of **genetically modified organisms** (GMOs) that can be potentially harmful to the environment and human health, the part of biotechnology that deals with GMOs is strictly regulated by biosafety laws and guidelines. The main thrust of this resource book is on the development and enforcement of such regulatory frameworks at domestic and international levels.

Biotechnology applications are developed by a collection of multidisciplinary research activities, commonly referred to as *enabling technologies*. Apart from fermentation and genetic engineering/recombinant DNA technology, other important enabling technologies are plant and animal cell culture technology and enzyme technology.

The basis of these enabling technologies are the scientific disciplines of molecular biology, genetics, microbiology, biochemistry, protein chemistry, chemical and

process engineering and computer science. An overview of important events in the development of modern molecular biology and recombinant DNA technology is provided in Table 1.2:

Table 1.2 | **An overview of recombinant DNA-based biotechnology**

Double helix structure of DNA is first described by Watson and Crick	1953
Cohen and Boyer, amongst others, develop genetic engineering	1973
The first human protein (somatostatin) is produced in a bacterium *(E. coli)*	1977
The first recombinant protein (human insulin) approved for the market	1982
Polymerase chain reaction (PCR) technique developed	1983
Launch of the Human Genome Project	1990
The first genome sequence of an organism *(Haemophilus influenzae)* is determined	1995
A first draft of the human genome sequence is completed	2000
Over 40 million gene sequences are deposited in GenBank, and genome sequences of hundreds of prokaryotes and dozens of eukaryotes are finished or in draft stage	2005

1.2 OVERVIEW OF APPLICATIONS OF BIOTECHNOLOGY

Since the advance of recombinant DNA technology, several techniques and applications have been developed that are benefiting humankind in the areas of agriculture, medicine, environment, industry and forensics. The following sections briefly describe some of these applications and their potential benefits to society.

1.2.1 Industry

Biotechnology can be used to develop alternative fuels; an example is the conversion of maize starch into ethanol by yeast, which is subsequently used to produce gasohol (a gasoline-ethanol mix). Bacteria are used to decompose sludge and landfill wastes (Soccol *et al.*, 2003). Through biotechnology, micro-organisms or

their enzymes can be adapted to convert biomass into feed stocks, or they can be used for manufacturing biodegradable plastics (bioplastics). Other organisms (micro-organisms, plants and mammals) are used as bioreactors for producing chemical compounds that are extracted from them and processed as drugs and other products. Plant and animal fibres are used for producing a variety of fabrics, threads and cordage. Biotechnology is applied to improve the quality and quantity of these products. Biopulping is a technique whereby a fungus is used to convert wood chips into pulp for papermaking (Gavrilescu and Chisti, 2005).

1.2.2 Health and medicine

In the area of health and medicine, biotechnology has numerous and important functions. Biotechnologies are used to develop diagnostic tools for identifying diseases.

Biotechnology is also used to produce more effective and efficient vaccines, therapeutic antibodies, antibiotics, and other pharmaceuticals. Biotechnology is a USD 70 billion a year industry that has produced several blockbuster drugs and vaccines, i.e. drugs with sales volumes exceeding USD 1 billion per year (Lawrence, 2007). Furthermore, there are more than 370 drug products and vaccines obtained through biotechnology currently in clinical trials, targeting more than 200 diseases including various cancers, Alzheimer's disease, heart disease, diabetes, multiple sclerosis, AIDS and arthritis (Sullivan *et al.*, 2008).

Through the biotechnology of gene therapy, scientists are making efforts at curing genetic diseases by attempting to replace defective genes with the correct version. A revolutionary strategy is being developed whereby staple foods such as potatoes, bananas, and others are used as delivery vehicles to facilitate the immunization of people in economically depressed regions of the world (Tacket, 2009).

1.2.3 Environment

Development and usage of alternative fuels that burn cleaner and improve air quality through reduced pollution of the environment is possible by biotechnological means. Micro-organisms are used to decompose wastes and clean up contaminated sites by the technology of bioremediation. The use of disease-resistant cultivars can make crop production less environmentally intrusive by reducing the use of agrochemicals (Chatterjee *et al.*, 2008).

1.2.4 Forensics

Since the DNA profile, i.e. the nucleotide sequence of the genome, is unique in every individual, it can be used as a powerful basis of identifying individuals in a population. DNA-based evidence is used in cases involving paternity disputes and family relationships. Furthermore, it is used in health care and judicial systems. In the judicial system, forensic experts use DNA profiling to identify suspects in criminal cases, especially when body fluids and other particles like hair and skin samples can be retrieved (Jobling and Gill, 2004).

1.2.5 Agriculture

Biotechnology can complement conventional breeding for crop and animal improvement. Instead of extensive re-arrangement of genes, as occurs in conventional breeding, biotechnology enables targeted gene transfer to occur. The genome of the recipient individual remains intact, except for the introduced gene (or genes), thus accelerating breeding programmes and the development of organisms with desirable characteristics. Furthermore, biotechnology enables gene transfer across natural breeding boundaries, overcoming mating barriers and creating a "universal gene pool" or "universal breeding population" accessible to all organisms. Likewise, it is possible to specifically introduce novel, desirable traits and characteristics into

existing species. This biotechnological application is used to improve the yield of crop and animal species and their product quality such as nutritional value and shelf life (Shewry *et al*, 2008). In addition to these benefits, this methodology reduces the need for agrochemicals by creating disease and pest-resistant species, thereby reducing environmental pollution from chemical runoff. Increased yields and higher food quality can contribute to reducing world hunger and malnutrition (FAO, 2004).

AGRICULTURAL BIOTECHNOLOGY
All biotechnological applications developed for a potential use in agriculture.

Several technologies in the field of **agricultural biotechnology** exist that do not rely on the creation of GMOs. Molecular techniques are being used to monitor breeding populations and to diagnose animals and plants infected with diseases. Micropropagation techniques are being widely used to generate clonal plant materials, allowing rapid large-scale clonal propagation of many plant species including trees. Biofertilizers and biopesticides can be applied in place of conventional fertilizer and pesticides to promote plant growth and health in an environmentally sustainable way (FAO, 2001).

To summarize, the field of biotechnology is very diverse, both in terms of methodologies and techniques applied and the potential applications and outcomes. Biotechnology has the potential to contribute to a worldwide sustainable development and the reduction of world hunger, including the branches of biotechnology concerned with agricultural research and development (FAO, 2004). Importantly, biotechnology is not only based on GMOs, but offers several important and well established techniques that are not dependent on or derived from genetic modifications. However, the focus of this publication is on GMOs and related products. Following an introduction to the molecular background and the scientific basis of GMO development and to the aims and prospects of this research, the main part of this book will introduce biosafety concepts related to the use of GMOs.

CHAPTER 2

STRUCTURE AND FUNCTION OF GENES

2.1 GENES AND HEREDITY

The study of genes and heredity is called **genetics**. Heredity phenomena have been of interest to humans since long before the underlying principles were scientifically investigated and understood. Ancient peoples were improving plant crops and domesticating animals by selecting desirable individuals for breeding. Genetics as a set of scientific principles and analytical procedures emerged in the 1860s when the Augustinian monk Gregor Mendel performed a set of experiments that revealed the existence of biological "factors" responsible for transmitting traits from generation to generation. These factors were later called genes, following the discovery of chromosomes and genetic linkage in the early twentieth century. Up to this point genetics looked at genes as abstract entities that somehow control hereditary traits. Through genetic analyses the inheritance of different genes was studied, but the physical and biochemical nature of the gene remained unknown. Further work revealed that chromosomes consist of DNA and protein, and subsequent studies allowed the conclusion that DNA is, in fact, the hereditary material (Morange and Cobb, 2000).

GENETICS
The science of genes and heredity.

DNA was thought to be a simple molecule, thus many scientists did not believe that it indeed carried and stored the information for an entire organism. How can such huge amounts of information be contained and passed on from one generation to the next? Clearly, the genetic material must have both the ability to encode specific information and the capacity to duplicate that information precisely during every cell division. What kind of molecular structure could allow such complex functions?

2.2 THE STRUCTURE OF DNA

Although the exact DNA structure was not known until 1953, its basic building blocks had been known for many years. It had been shown that DNA is composed of four basic molecules called *nucleotides*, which are identical except that each contains a different nitrogen-containing base. Each nucleotide is made up of a phosphate group, a sugar (of the deoxyribose type), and one of the four bases. The four bases are adenine (A), guanine (G) (the purines) and cytosine (C) and thymine (T) (the pyrimidines; see also Figure 2.1).

DNA STRUCTURE
In 1953, the structure of DNA was deciphered and found to be a double helix. This model offered possible explanations for the processes of DNA replication and gene expression.

In 1953 James Watson and Francis Crick were the first to succeed in putting the building blocks together and came up with a reasonable **DNA structure**. They used DNA X-ray diffraction patterns produced by Rosalind Franklin and Maurice Wilkins and data from Erwin Chargaff. The X-ray data showed the DNA molecule to be long, thin and helical (spiral-like) in shape.

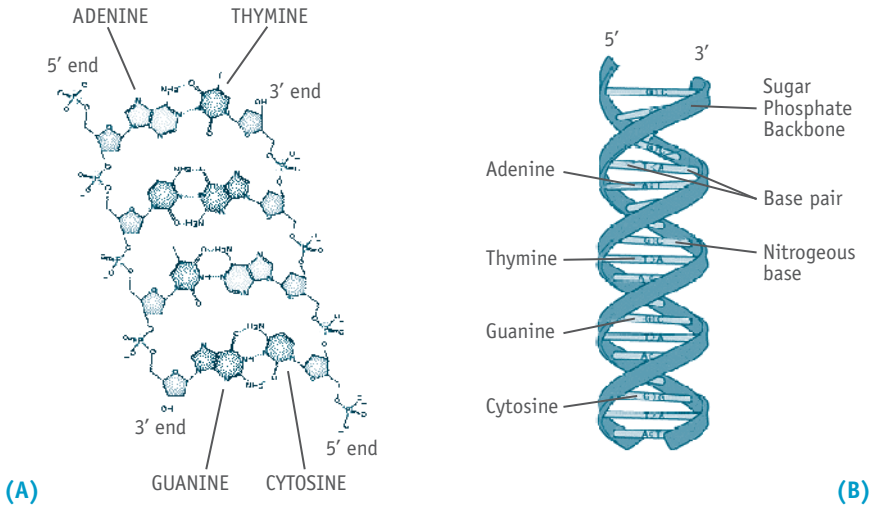
Chargaff had established certain empirical rules about the amounts of each component of DNA:

- The total amount of pyrimidine nucleotides (T + C) always equals the total number of purine nucleotides (A + G).
- The amount of T always equals the amount of A, and the amount of C always equals the amount of G. But the amount of A + T is not necessarily equal to the amount of G + C.

The structure that Watson and Crick derived from these clues is a double helix (Figure 2.1). Each helix is a chain of nucleotides held together by phosphodiester bonds, in which a phosphate group forms a bridge between -OH groups on two adjacent sugar residues. The two DNA chains (helices) are running in an antiparallel direction and are held together by hydrogen bonds between opposing bases, thus forming a double helix. Each base pair (bp) consists of one purine and one pyrimidine base, paired according to the following rule: G pairs with C, and A pairs with T (Watson *et al.*, 2008).

Figure 2.1 | **The structure of DNA**

In part (A), the four bases, the pairing of the bases and the connection of the bases through the sugarphosphate-backbone are depicted. Note that the two DNA strands are held together through base pairing and are running in opposite direction, labelled 3′ and 5′ end respectively (read: three prime and five prime). In part (B), a schematic drawing of the actual DNA double helix structure is depicted, containing the same elements in simplified form and labelling as in (A).



Elucidation of the structure of DNA caused a lot of excitement in the scientific community for two major reasons. First, the structure suggests an obvious way in which the molecule can be duplicated, or replicated, since each base can specify its complementary base by hydrogen bonding. Thus each strand can serve as a template for the synthesis of a complementary strand. Second, the structure suggests that the sequence of nucleotide pairs in DNA is dictating the sequence of amino acids in a protein encoded by a gene. In other words, some sort of genetic code may comprise information in DNA as a sequence of nucleotide pairs, which can be translated into the different language of amino acid sequence in protein.

2.3 THE FLOW OF GENETIC INFORMATION: THE CENTRAL DOGMA

THE CENTRAL DOGMA
Francis Crick proposition that DNA is transcribed to a messenger molecule (mRNA) which is subsequently translated to protein: DNA makes RNA makes protein.

In the early 1950s, Francis Crick suggested that there is a unidirectional flow of genetic information from DNA through ribonucleic acid (RNA) to protein, i.e. "DNA makes RNA makes protein". This is known as **the central dogma** of molecular biology, since it was proposed without much evidence for the individual steps. Now these steps are known in detail: DNA is transcribed to an RNA molecule (messenger RNA [mRNA]), that contains the same sequence information as the template DNA, and subsequently this RNA message is translated into a protein sequence according to the genetic code (Miller *et al.*, 2009).

2.4 THE GENETIC CODE

THE GENETIC CODE
The relation between the sequence of bases in DNA, in 64 possible nucleotide triplet combinations, and the sequence of amino acids in protein.

The basic building blocks of DNA are the four nucleotides; the basic building blocks of proteins are the amino acids, of which there are 22 that naturally occur in proteins (the so-called proteinogenic amino acids). **The genetic code** is the correspondence between the sequence of the four bases in nucleic acids and the sequence of the 22 amino acids in proteins. It has been shown that the code is a triplet code, where three nucleotides (one codon) encode one amino acid. Since

BOX 2.1

RNA

The ***ribonucleic acids (RNA)*** are an important class of molecules in the flow of genetic information. Some viruses even use RNA, instead of DNA, to carry their genetic information. All other organisms that use DNA as the genetic material must first transcribe their genetic information into RNA, in order to render the information accessible and functional.

RNA is similar in composition to DNA. It is a long linear molecule (polymer) that is made up of a limited number of monomers, the nucleotides. As in DNA, each nucleotide is composed of a sugar, a phosphate, and a base. The sugar, however, is ribose instead of deoxyribose as seen with DNA, hence the names ribo- and deoxyribonucleic acids. Unlike DNA, RNA molecules are usually single stranded and do not form double helices. RNA molecules are made up of the same bases as DNA, except that the DNA base thymine (T) is replaced by uracil (U) in RNA.

The cell contains different kinds of RNA, most importantly messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). These three RNA classes correspond to the three basic roles RNA plays in the cell.

First, RNA serves as the intermediary in the flow of information from DNA to protein. The DNA is transcribed (copied) into mRNA via an enzyme (RNA polymerase) and subsequently the mRNA is translated into protein. In the latter process, translation of mRNA to protein, tRNA and rRNA play important roles. tRNA molecules serve as adaptors that translate the information in the nucleic acid sequence of mRNA into the sequence of amino acids, the constituents that make up a protein. Finally, the rRNA molecules are the major functional components of the molecular machines, the so-called ribosomes, which carry out the translation process.

RNA
Acronym for ribonucleic acid, the second class of nucleic acids in the cell besides DNA. RNAs occupy several crucial functions in the flow of genetic information from DNA to protein.

there are only 22 amino acids to be specified and 64 different codons (4^3 =64), most amino acids are specified by more than one codon and the genetic code is said to be degenerate, or to have redundancy. The genetic code has colinearity, which means that the order of the bases in the DNA corresponds directly to the order of amino acids in the protein (Watson *et al.*, 2008).

Clearly, if the genetic code is to be read as we would read a sentence in a book, we need to know where to start and stop. The codon AUG serves as a start signal, encoding the amino acid methionine, which is therefore the first amino acid incorporated into all proteins. However, methionine is also found elsewhere, not only at the beginning. Therefore, the translational machinery has to find the correct methionine codon to start and not just any given AUG codon anywhere in the gene sequence. This process is facilitated by sequences surrounding the initiation AUG codon. These sequences are therefore highly important for the translation process. The end of the translated region is specified by one of three codons which encode "stop". These are UAA, UAG and UGA. If **mutations**, i.e. unintended changes in the DNA sequence, take place that create one of the stop codons instead of an amino acid encoding codon, the results may be severe as the resultant protein will be shorter than intended. Such proteins are referred to as being truncated, and are very likely non-functional. Other mutations alter one codon to another, resulting in the replacement of the original amino acid by a different one, which can have severe or negligible effects, depending on the importance of the amino acid, for the entire protein. The addition or deletion of a single nucleotide can also have a severe effect, since all following codons will be shifted by one nucleotide, resulting in a very different message – a so-called frameshift mutation. The region between the start-methionine and the first stop codon is referred to as the open reading frame (ORF).

MUTATION
Random changes in DNA sequence, induced by replication errors, mutagenic substances or physical factors such as UV light and radioactive irradiation.

Finally, the genetic code is virtually universal, i.e. it is the same in all organisms living on this planet. Genes taken from plants can be decoded by animal cells, while genes from prokaryotes can be decoded by eukaryotic systems, and vice versa.

Without such a universal nature of the code, genetic manipulation and genetic engineering would be much more difficult (Voet and Voet, 2004).

2.5 THE GENE

Historically, a **gene** is defined as a heritable unit of phenotypic variation. From a molecular standpoint, a gene is the linear DNA sequence required to produce a functional RNA molecule, or a single transcriptional unit (Pearson, 2006). Genes can be assigned to one of two broad functional categories: structural genes and regulatory genes. It is the function of the end product of a gene that distinguishes structural and regulatory genes.

GENE
Broadly defined as a sequence of DNA encoding a functional product. This includes the coding region itself as well as all assoctiated regulatory regions.

- *Structural genes* code for polypeptides or RNAs needed for the normal metabolic activities of the cell, e.g. enzymes, structural proteins, transporters, and receptors, among others.
- *Regulatory genes* code for proteins whose function is to control the expression of structural genes. With regard to molecular composition both classes of genes are similar.

A gene usually occupies a defined location on a chromosome, of which there are 46 in every human cell and which contain the entire human **genome** (see below). The exact chromosomal gene location is defined by specific sequences for the start and termination of its transcription. Each gene has a specific effect and function in the organism's morphology or physiology, can be mutated (i.e. changed), and can recombine with other genes. It is a store of information (in the form of nucleotide base sequence); consequently it does not initiate any action, but is acted upon, e.g. during the process of gene expression. The complete set of genes of an organism, its genetic constitution, is called the genotype. The human genome, for example, contains an approximate number of 25 000 protein-coding genes. The physical manifestation, or expression, of the

GENOME
The complete set of genetic information of an organism encoded in its DNA.

PHENOTYPE
All observable characteristics and traits of an organism. The phenotype is the result of the organism's genotype and environmental influences.

genotype is the **phenotype** (i.e. the organism's morphology and physiology). If a particular characteristic, such as brown eye colour, is part of an organism's phenotype, one can conclude that the individual carries the gene(s) for that characteristic. If, however, a particular characteristic is not expressed, one cannot implicitly conclude that the particular gene is absent because expression of that gene might be repressed. Different varieties of the same gene, resulting in different phenotypic characteristics, are called **alleles** (Griffiths *et al.*, 2007).

ALLELE
Different versions of the same gene. In a population, usually many versions of the same gene can be found.

Genes may be located on either strand of the double-stranded DNA. But, regardless of which strand contains a particular gene, all genes are read in a 5′ to 3′ direction, and the strand containing the particular gene is referred to as the sense or coding strand.

CHROMOSOME
A single DNA molecule, associated with specific proteins. The storage form of DNA within the cell.

As stated above, every cell of a human body, except germ line cells, contains 46 **chromosomes**. From each parent, we inherit 23 chomosomes, representing the complete genome. Thus, each body cell is *diploid*, i.e. contains two copies of the human genome and likewise two copies (alleles) of each gene.

The haploid set of the human genome (23 chromosomes), consists of approximately 3 200 megabases (Mb; 1 Mb = 10^6 bp) and contains an estimated number of 20 000 to 25 000 protein-coding genes (International Human Genome Sequencing Consortium, 2004). In fact, protein-coding DNA sequences only represent approximately 1.5 percent of the total genome; the remaining majority of DNA represent regulatory sequences, RNA encoding genes, or simply DNA sequences that have not yet been assigned to a certain function (sometimes inappropriately referred to as "junk DNA"). Interestingly, the estimated number of proteins is somewhat higher than the number of genes, due to alternative splicing (see 2.6 and 2.9.1) and other variations in gene expression.

In comparison, the genome of *E. coli*, a widely used model bacterium, consists of one chromosome of 4.6 Mb in size, encoding approximately 4 400 genes in

total. The genome of *Arabidopsis thaliana*, probably the most important model plant, consists of five chromosomes, of 157 Mb in size and encodes approximately 27 000 genes. Importantly, there is no straight connection between genome size, number of genes and organism complexity; some plants, vertebrates and even protozoans (single-cell organisms) have significantly larger genomes than the human genome (Patrushev and Minkevich, 2008).

2.6 THE ARRANGEMENT AND LAYOUT OF GENES

In **eukaryotic organisms** each cell contains more than one DNA molecule packaged into individual chromosomes; a diploid human cell, as stated, contains 46 chromosomes. Along the length of each DNA molecule/chromosome one can find thousands of genes, with more or less random spacing. In bacteria, one can frequently find clusters of genes that are related, in the sense that the proteins encoded by these genes are required in the same metabolic pathway. Therefore, as the cell needs all the gene products more or less simultaneously in order to keep that pathway running, it is appropriate for the cell to arrange these genes in clusters and employ a mechanism to express them together. These clusters of genes are known as operons; the most studied **operon** is the lactose operon in *E. coli*. This operon contains three genes which are adjacent on the DNA and are required for the utilization of lactose as a metabolic energy source in the cell. The operon also contains all the control sequences (repressor, promoter and operator, see Figure 2.2) needed to ensure efficient expression of the genes as an ensemble (Reznikoff, 1992). Operons do not occur in higher organisms but related genes are sometimes found in clusters as well, and comparable regulatory mechanisms are found.

EUKARYOTES
All organisms that possess a cellular structure, called nucleus, in which the DNA is contained within each cell. This includes all organisms except bacteria and archaebacteria, which do not posess a nucleus and are referred to as Prokaryotes.

OPERON
An arrangement of genes and certain regulatory regions to ensure expression of the genes as an ensemble in a controlled manner.

Many genes in eukaryotes have a distinctive structural feature: the nucleotide sequence contains one or more intervening segments of DNA that do not code for the amino acid sequence of the protein. These non-translated sequences interrupt the otherwise co-linear relationship between the nucleotide sequence of the gene

and the amino acid sequence of the protein it encodes. Such non-translated DNA segments in genes are called introns. The pieces that constitute mature mRNA, and therefore ultimatively for protein, are referred to as exons. During and after transcription the exons are spliced together from a larger precursor mRNA that contains, in addition to the exons, the interspersed introns. The number of exons that constitute a final mRNA molecule depends on the gene and the organism, but can range from as few as one to as many as fifty or more. The origin of **intron/exon structure** is a matter of scientific debate. To date it is not clear whether it predated the divergence of eukaryotes and prokaryotes with the subsequent loss of introns in prokaryotes, or if introns and the splicing mechanism evolved in eukaryotes after their evolutionary separation from prokaryotes (Mattick, 1994).

INTRONS AND EXONS
In eukaryotes, genes often consist of coding regions (exons) with interspersed non-coding regions (introns) which are removed during the process of gene expression.

In addition to introns and exons, the structural features of the eukaryotic gene include regulatory elements, a promoter region, a transcription start site and a transcription termination site (Figure 2.2). Specific proteins in the cell nucleus, the cellular compartment where DNA is stored, can bind to regulatory element sequences of a gene, thus controlling the expression of that gene. The promoter region is the sequence of the gene where the transcription machinery (the assembly of proteins required for transcription) binds to the DNA in order to start transcription to RNA. The start site indicates to the transcription machinery where to start and the termination site indicates were to stop transcription of the gene.

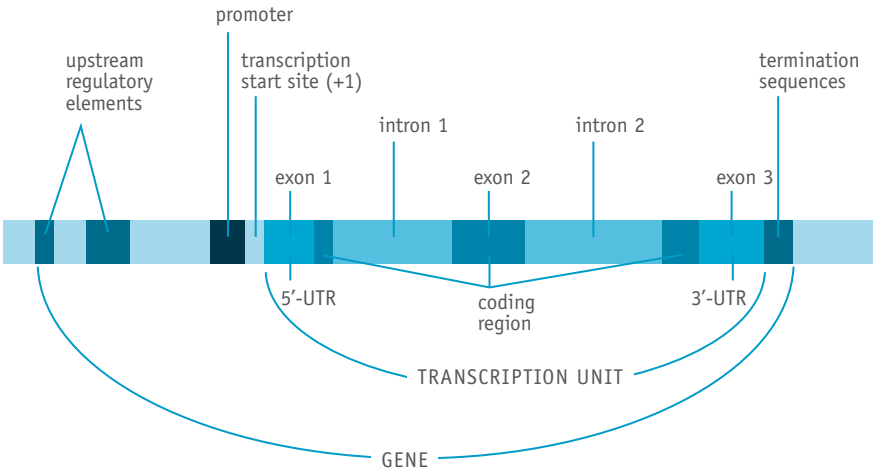
2.7 GENE EXPRESSION

GENE EXPRESSION
The process of converting the information stored in a gene to a functional product. Correct regulation of gene expression is crucial for the correct development and function of an organism.

Genes exert their function through a process called **gene expression**, a process by which heritable information from a gene, encoded on DNA, is transformed into a functional gene product, such as protein or RNA (some genes code for functional RNA molecules, such as tRNA and rRNA). Genes are expressed by being first transcribed into RNA, and may then subsequently be translated into protein. A cell employs many different mechanisms to regulate gene expression. Gene

Figure 2.2 | **A general structural arrangement of the different components making up a eukaryotic gene**

Upstream regulatory elements (enhancers) and the promoter are required for regulation and initiation of transcription. Exons, which constitute the actual protein-coding regions, and interspersed introns are indicated. The 5′ and 3′ untranslated regions (UTRs) are mRNA sequences that do not encode protein, but are required for a correct translation process. Transcription start and termination sites are also indicated.



expression can be regulated at many different levels, from DNA transcription, pre-mRNA processing, mRNA stability and efficiency of translation up to protein modification and stability. Thus, a cell can precisely influence the expression level of every gene, and studying and predicting gene expression levels is a difficult task. Nevertheless, this is especially important for biotechnological applications, since it is desirable to precisely define the expression levels of introduced genes in transgenic organisms. In the following section, the major processes of gene expression will be introduced.

2.7.1 Transcription and translation

mRNA
Messenger RNA, the molecule that DNA is transcribed to and that is subsequently translated to protein.

The first step in gene expression is transcription, namely the production of a single-stranded RNA molecule known as mRNA in the case of protein-coding genes. The nucleotide sequence of the **mRNA** is complementary to the DNA from which it was transcribed. In other words, the genetic messages encoded in DNA are copied precisely into RNA. The DNA strand whose sequence matches that of the RNA is known as the *coding strand* and the complementary strand on which the RNA was synthesized is the *template strand*.

TRANSCRIPTION
The process of transferring genetic information from DNA to an RNA molecule. Performed by the enzyme RNA Polymerase.

Transcription is performed by an enzyme called RNA polymerase, which reads the template strand in 3′ to 5′ direction and synthesizes the RNA from 5′ to 3′ direction. To initiate transcription, the polymerase first recognizes and binds a promoter region of the gene. Thus a major regulatory mechanism of gene expression is the blocking or sequestering of the promoter region. This can be achieved either by tight binding of repressor molecules that physically block the RNA polymerase, or by spatially arranging the DNA so that the promoter region is not accessible (Thomas and Chiang, 2006).

In eukaryotes, transcription occurs in the nucleus, where the cell's DNA is sequestered. The initial RNA molecule produced by RNA polymerase is known as the primary transcript and must undergo post-transcriptional modification before being exported to the cytoplasm for translation. The splicing of introns present within the transcribed region is a modification unique to eukaryotes. The splicing reaction offers various possibilities for regulating and modulating gene expression in eukaryotic cells.

Following transcription and post-transcriptional mRNA processing, the mRNA molecule is ready for translation. In eukaryotes, the mRNA must first be transported from the nucleus to the cytoplasm, whereas in prokaryotes no nucleus exists and transcription and translation take place in the same compartment.

Translation is the process by which a mature mRNA molecule is used as a template for synthesizing a protein. Translation is carried out by the ribosome, a large macromolecular complex of several rRNA and protein molecules. Ribosomes are responsible for decoding the genetic code on the mRNA and translating it into the amino acid sequence of proteins. Likewise, they are catalysing the chemical reactions that add new amino acids to a growing polypeptide chain by the formation of peptide bonds (Ramakrishnan, 2002).

TRANSLATION
The process of using the genetic information from an mRNA molecule to synthesize a protein molecule. Performed by the ribosome.

The genetic code on the mRNA is read three nucleotides at a time, in units called codons, via interactions of the mRNA with specialized RNA molecules called transfer RNA (tRNA). Each tRNA has three unpaired bases, known as the anticodon, that are complementary to the codon it reads. The tRNA is also covalently attached to the amino acid specified by its anticodon. When the tRNA binds to its complementary codon in an mRNA strand, the ribosome ligates its amino acid cargo to the growing polypeptide chain. When the synthesis of the protein is finished, as encoded by a stop-codon on the mRNA, it is released from the ribosome. During and after its synthesis, the new protein must fold to its active three-dimensional structure before it can carry out its cellular function (Voet and Voet, 2002).

A single mRNA molecule can be translated several times and thus produce many identical proteins, depending on its half-life in the cell, i.e. the average time it remains within the cell before it is degraded.

2.8 REGULATION OF GENE TRANSCRIPTION

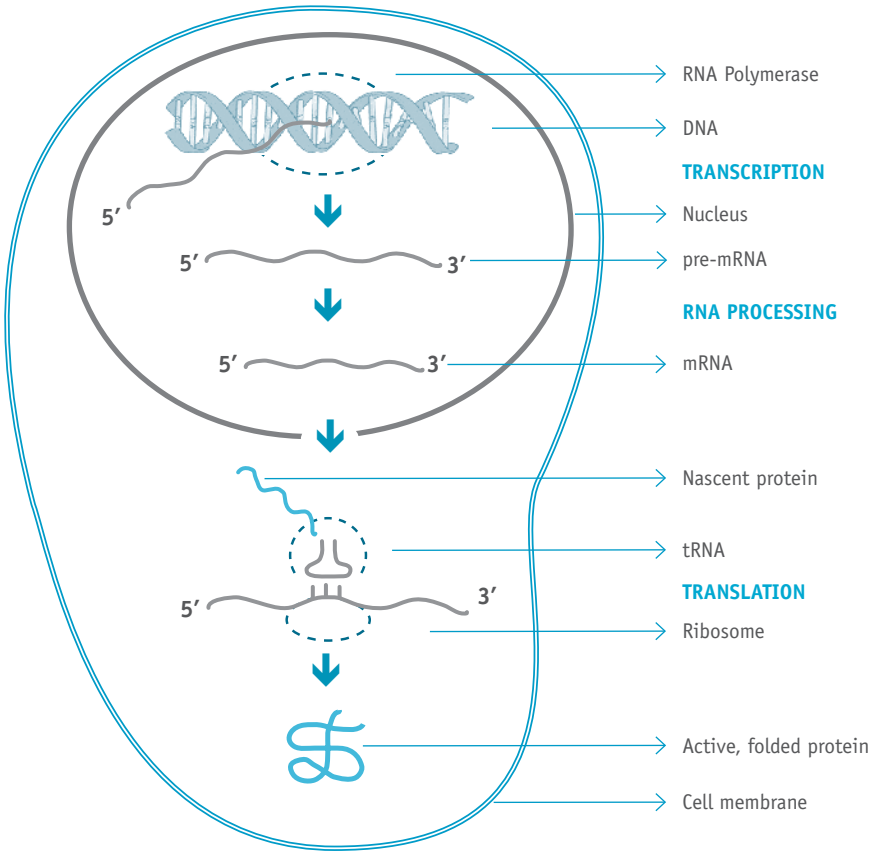
2.8.1 Promoters

PROMOTER
A DNA sequence associated with a gene that is responsible for recruiting the enzyme machinery required for the expression of that gene.

The promoter region of a gene is usually several hundred nucleotides long and immediately upstream from the transcription initiation site. The **promoter** constitutes the binding site for the enzyme machinery that is responsible for the transcription of DNA to RNA, the RNA polymerase. In eukaryotic cells several RNA polymerases

Figure 2.3 | Transcription and Translation

In the nucleus, DNA is transcribed to a pre-mRNA molecule by RNA polymerase. The pre-mRNA is processed, e.g. by intron excision, to the mature mRNA. The mRNA is exported to the cytoplasm and translated into protein, which is accomplished by ribosomes and tRNA that together decode the genetic code into amino acid sequence. Following translation, the synthesized protein adopts its correct 3-dimensional shape and is ready to perform its cellular function.



are present, the most prominent one, that is responsible for the transcription of protein-coding genes, being RNA polymerase II. There are different types of promoters for different RNA polymerases. Promoters for RNA polymerase II, the polymerase that transcribes protein-coding genes into mRNA, often contain the consensus sequence 5'-TATA-3', 30 to 50 bp upstream of the site at which transcription begins. Many eukaryotic promoters also have a so-called CAAT box with a GGNCAATCT consensus sequence centred about 75 bp upstream of the initiation start site (with N representing any of the four bases). RNA polymerases I and III are mainly responsible for the transcription of RNA molecules that possess an intrinsic function as catalytic or structural molecules, such as tRNA and rRNA, and that are not translated into proteins (Okkema and Krause, 2005). In general, the promoter region has a high importance for the regulation of expression of any gene. This concept will come up again later on in this module when the production of transgenic animals is introduced. Careful choice of promoters to drive gene expression in transgenic organisms is very important to ensure the transgenic organism possesses the desired characteristics.

2.8.2 Enhancers

ENHANCERS
DNA sequences that influence the expression of a gene, often over long distances of DNA sequence.

Enhancers were first described as sequences that increase transcription initiation but, unlike promoters, were not dependent on their orientation or the distance from the transcription start site. It is now apparent that **enhancers** are generally short sequences (less than 20 to 30 bp) that bind specific transcription factors, which then facilitate the assembly of an activated transcriptional complex (i.e. the RNA polymerase) at the promoter. Most enhancers function both on the coding and non-coding strand of the DNA (i.e. in either orientation), can act up to several thousand bps distant from their target promoter, and are a rather unspecific form of regulatory element (Visel *et al.*, 2007). This implies that an enhancer element may influence several, possibly very distant, promoters. Most enhancers are only active in specific cell types and therefore play a central role in regulating tissue specificity of gene expression. Some regulatory elements bind transcription factors that act to reduce

the efficiency of transcriptional initiation, and many genes contain a combination of both positive and negative upstream regulatory elements, which then act in concert on a single promoter. This allows gene expression to be controlled very precisely in a temporal and spatial manner with regard to cell type, developmental stage and environmental conditions. Mutations of promoters or enhancers can significantly alter the expression pattern, but not the structure of a particular gene product.

2.8.3 Operators

Operators are nucleotide sequences that are positioned between the promoter and the structural gene. They constitute the region of DNA to which repressor proteins bind and thereby prevent transcription. Repressor proteins have a very high affinity for operator sequences. Repression of transcription is accomplished by the repressor protein attaching to the operator sequence downstream of the promoter sequence (the point of attachment of the RNA polymerase). The enzyme must pass the operator sequence to reach the structural genes start site. The repressor protein bound to the operator physically prevents this passage and, as a result, transcription by the polymerase cannot occur (Reznikoff, 1992). Repressor proteins themselves can be affected by a variety of other proteins or small molecules, e.g. metabolites, that affect their affinity for the operator sequence. This allows a further level of gene expression regulation to be accomplished.

2.8.4 Attenuators

The attenuator sequences are found in bacterial gene clusters that code for enzymes involved in amino acid biosynthesis. Attenuators are located within so-called leader sequences, a unit of about 162 bp situated between the promoter-operator region and the start site of the first structural gene of the cluster. Attenuation decreases the level of transcription approximately 10-fold. As the concentration of an amino acid in the cell rises and falls, attenuation adjusts the level of transcription to accommodate the changing levels of the amino acid. High concentrations of the amino acid result in low levels of transcription of the structural genes, and low concentrations of

the amino acid result in high levels of transcription. Thus the biosynthesis of an amino acid can be linked to the actual concentration of that amino acid within the cell. Attenuation proceeds independently of repression, the two phenomena are not dependent on each other. Attenuation results in the premature termination of transcription of the structural genes (Yanofsky *et al.*, 1996).

Several other regulatory elements have been described that regulate gene expression at the level of transcription. In general, the interplay of all involved factors and sequences is, in most cases and especially in eukaryotes, very complex and not entirely understood. The **expression level** of a gene is therefore the net result of all stimulating and repressing factors acting on it (Watson, 2008). This combinatorial system of positive and negative influences allows the fine-tuning of gene expression and needs to be carefully considered when designing transgenic organisms.

EXPRESSION LEVEL
The frequency with which a given gene is transcribed and translated, i.e. how much of a given gene product is produced over time.

2.9 REGULATORY mRNA SEQUENCES

In the preceding paragraph, DNA sequences were described that regulate transcription of DNA to an mRNA transcript. This transcript, sometimes referred to as pre-mRNA, contains a variety of sequences in addition to the protein-coding sequences. This includes 5′ and 3′ untranslated sequences which are important in the regulation of translation, and introns (in the case of eukaryotes) which need to be excised before the process of translation can take place. In eukaryotes, processing of a pre-mRNA to a mature mRNA that is ready for translation takes place in the same compartment as transcription, the nucleus.

2.9.1 Introns and splice junctions

In eukaryotic pre-mRNA processing, intervening sequences (introns) that interrupt the coding regions are removed (spliced out), and the two flanking protein-coding exons are joined. This splicing reaction occurs in the nucleus and requires the intron to have a GU-dinucleotide sequence at its 5′-end, an AG-dinucleotide at its

3′-end, and a specific branch point sequence. In a two-step reaction, the intron is removed as a tailed circular molecule, or lariat, and is subsequently degraded. This splicing reaction is performed by RNA-protein complexes known as snRNPs (small nuclear ribonucleoproteins). The snRNPs bind to the conserved intron sequences to form a machinery called spliceosome, in which the cleavage and ligation reactions take place (Matthew *et al.*, 2008).

2.9.2 5′ Untranslated sequences

During the processing of precursor mRNA in the nucleus, the 3′ terminus as well as introns are removed. In addition, shortly after initiation of mRNA transcription, a methylguanylate residue is added to the 5′ end of the primary transcript. This 5′ "cap" is a characteristic feature of every mRNA molecule, and the transcriptional start or initiation site is also referred to as the capping site. The 5′ UTR extends from the capping site to the beginning of the protein coding sequence and can be up to several hundred bps in length. The **5′ UTRs** of most mRNAs contain the consensus sequence 5′ –CGAGCCAUC-3 involved in the intiation of protein synthesis (i.e. translation). In addition, some 5′ UTRs contain "upstream AUGs" that may affect the initiation of protein synthesis and thus could serve to control expression of selected genes at the translational level (Hughes, 2006).

5′UNTRANSLATED REGIONS
Sequence in mRNA, upstream of the coding region, that regulate initiation of translation.

2.9.3 3′ Untranslated sequences and transcriptional termination

The 3′ end of a mature mRNA molecule is created by cleavage of the primary precursor mRNA and the addition of a several hundred nucleotide long polyadenylic acid (poly-A) tail. The site for cleavage is marked by the sequence 5′ AAUAAA 3′ some 15 to 20 nucleotides upstream and by additional uncharacterized sequences 10 to 30 nucleotides downstream of the cleavage site. The region from the last protein codon to the poly-A addition site may contain up to several hundred nucleotides

of a **3′ UTR**, which includes signals that affect mRNA processing and stability. Many mRNAs that are known to have a short half life contain a 50 nucleotide long AU-rich sequence in the 3′ UTR. Removal or alteration of this sequence prolongs the half life of mRNA, suggesting that the presence of AU-rich sequences in the 3′ UTR may be a general feature of genes that rapidly alter the level of their expression. In general, the half-life of an mRNA indicates the average time that an mRNA molecule persists in the cell and thus can be translated before it is degraded. The mRNA half-life is therefore an important variable for the level of gene expression (Gray and Wickens, 1998).

3′ UNTRANSLATED REGION
Sequence in mRNA, downstream of the coding region, that regulates mRNA processing and stability.

2.9.4 Regulation of gene expression

Regulation of gene expression refers to the all processes that cells employ to convert the information carried by genes into gene products in a highly controlled manner. Although a functional gene product may be RNA or protein, the majority of known regulatory mechanisms affect the expression level of protein coding genes. As mentioned above, any step in the process of gene expression may be modulated, from transcription, to RNA processing, to translation, to post-translational modification of the protein. Highly sophisticated **gene expression regulatory systems** allow the cell to fine-tune its requirements in response to environmental stimuli, developmental stages, stress, nutrient availability etc. (Nestler and Hyman, 2002; Watson *et al.*, 2008).

REGULATION OF GENE EXPRESSION
All mechanisms employed by a cell/organism to regulate the expression level of its genes, in response to internal or external stimuli or developmental stages.

To conclude, this chapter has provided an overview of genes, gene expression and hereditary phenomena. Although this text offers only a brief introduction to the topic, it should have become clear that correct gene expression is based on a highly complicated network and interplay of numerous factors, and a complete comprehension of these networks is only beginning to emerge. However, a good understanding of the basic principles is required to follow and understand biotechnological applications and developments, as well as the associated current

limits and difficulties of this technology. The following chapter will introduce techniques and scientific concepts that are more specific to and highly important for modern, applied biotechnology, especially in the field of GMOs. The chapter is based on the principles of DNA structure, genes, and gene expression that have been described in this chapter.

CHAPTER 3

VECTORS AND PROMOTERS

3.1 RECOMBINANT DNA TECHNOLOGY – AN OVERVIEW

Following the elucidation of the DNA structure and the genetic code, it became clear that many biological secrets were hidden in the sequence of bases in DNA. Technical and biological discoveries in the 1970s led to a new era of DNA analysis and manipulation. Key among these was the discovery of two types of enzymes that made **DNA cloning** possible: cloning, in this sense, refers to the isolation and amplification of defined pieces of DNA. One enzyme type, called ***restriction enzymes,*** cut the DNA from any organism at specific sequences of a few nucleotides, generating a reproducible set of fragments. Restriction enzymes occur naturally in many bacteria, where they serve as defence mechanisms against bacteriophage (viruses infecting bacteria) infection by cutting the bacteriophages genome upon its entry into the cell. The other enzyme type, called *DNA ligases,* can covalently join DNA fragments at their termini that have been created by restriction enzymes. Thus, ligases can insert DNA restriction fragments into replicating DNA molecules such as plasmids (bacterial, circular DNA molecules), resulting in recombinant DNA molecules. The recombinant DNA molecules can then be introduced into appropriate host cells, most often bacterial cells. All descendants from such a single cell, called a clone, carry the same recombinant DNA molecule (Figure 3.1). Once a clone of

DNA CLONING
The isolation and amplification of defined sequences of DNA.

RESTRICTION ENZYME
Enzymes, naturally present in bacteria, that cut DNA at defined sequences.

cells bearing a desired segment of DNA has been isolated, unlimited quantities of this DNA sequence can be prepared (Allison, 2007). Furthermore, in case the DNA fragment contains protein-coding genes, the recombinant DNA molecule introduced into a suitable host can direct the expression of these genes, resulting in the production of the proteins within the host. These developments, DNA cloning and the production of **recombinant proteins**, were major breakthroughs in molecular biology and set the stage for modern biological research.

RECOMBINANT PROTEINS
Proteins produced with the aid of recombinant DNA technology.

3.2 VECTORS

A vector is a DNA molecule which can replicate in a suitable host organism, and into which a fragment of foreign DNA can be introduced. Most vectors used in molecular biology are based on bacterial plasmids and bacteriophages (bacteria-infecting viruses).

VECTOR
In molecular biology, a vector is a DNA molecule that can take up foreign DNA fragments and can be used to amplify and transfer this DNA to a suitable host.

Vectors need to have the following characteristics:

- Possess an origin of replication (ori), which renders the vector capable of autonomous replication independent of the host genome.
- Have a site (or sites) which can be cleaved by a restriction enzyme, where the foreign DNA fragment can be introduced.
- Contain convenient markers for identifying the host cell that contains the vector with the inserted DNA of interest. A common selection marker is an antibiotic resistance gene. If the host bacteria cells contain the vector then the bacteria will grow in the presence of that antibiotic, whereas growth of bacteria without the plasmid is restricted.

In addition to the above-listed features, the vector should be easily introducible into the host organism where it has to replicate and produce copies of itself and the foreign DNA. Furthermore, it should be feasible to easily isolate the vector from the host cell (Watson, 2008).

3.3 TYPES OF CLONING VECTORS

3.3.1 Plasmids

Plasmids are circular, double-stranded DNA molecules that are independent from a cell's chromosomal DNA. These extrachromosomal DNAs occur naturally in bacteria and in the nuclei of yeast and some higher eukaryotic cells, existing in a parasitic or symbiotic relationship with their host cell. Most naturally occurring **plasmids** contain genes that provide some benefit to the host cell, fulfilling the plasmid's portion of a symbiotic relationship. Some bacterial plasmids, for example, encode enzymes that inactivate antibiotics. Therefore, a bacterial cell containing such a plasmid is resistant to the antibiotic, whereas the same type of bacterium lacking the plasmid is killed. Plasmids range in size from a few thousand bps to more than 100 kilobases (kb).

PLASMIDS
Circular, extrachromosomal DNA molecules, usually derived from bacteria, capable of autonomous replication and maintenance within the cell.

The plasmids most frequently used in recombinant DNA technology are derived from and replicate in *E. coli* (Jana and Deb, 2005). In general, these plasmids have been modified to optimize their use as vectors in DNA cloning. One such modification, for example, is the reduction in size to approximately 3 kb, which is much smaller than that of naturally occurring *E. coli* plasmids. In addition, most plasmids contain a multiple cloning site (MCS), a short sequence of DNA containing many restriction enzyme sites close together. Thus, many different restriction enzymes can be used for the insertion of foreign DNA fragments. In addition to antibiotic resistance genes, many modern plasmid vectors also contain a system for detecting the presence of a recombinant insert, such as the blue/white β-galactosidase system that allows simple visual screening of bacterial clones.

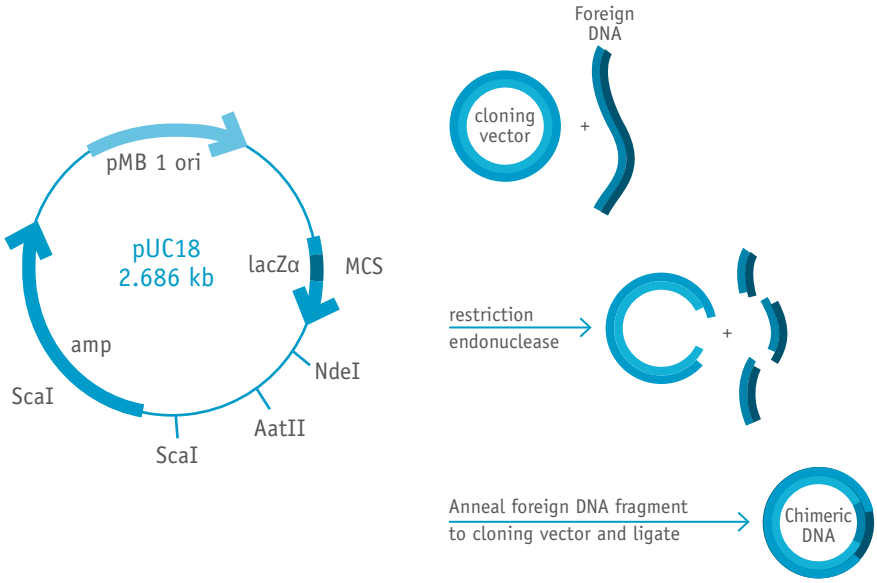
3.3.2 Bacteriophages

BACTERIOPHAGES
Viruses that infect bacteria. Bacteriophages can be manipulated and used as DNA cloning vectors.

Bacteriophages, or phages, are viruses that infect bacteria. They can display either lytic life cycles, leading to the death of the host bacterium and release of

Figure 3.1 | **A typical plasmid cloning vector and the principle of DNA cloning**

The pUC18 plasmid is a frequently used plasmid for DNA cloning. The plasmid size, ampicillin resistance gene (amp), origin of replication (ori) and multiple cloning site (MCS) are indicated. On the right hand side, the overall principle of DNA cloning is depicted.



new phage particles, or more complex lysogenic cycles during which the phage genome is integrated into the bacterial genome. One of the best studied phages is bacteriophage λ (Lambda) whose derivatives are commonly used as cloning vectors (Chauthaiwale *et al.*, 1992). The λ phage particle consists of a head containing the 48.5 kb double-stranded DNA genome, and a long flexible tail. During infection, the phage binds to certain receptors on the outer membrane of *E. coli* and subsequently injects its genome into the host cell through its tail. The phage genome is linear

and contains single-stranded ends that are complementary to each other (the so-called cos ends). Due to the complementarity, the cos ends rapidly bind to each other upon entry into the host cell, resulting in a nicked circular genome. The nicks are subsequently repaired by the cellular enzyme DNA ligase. A large part of the central region of the phage genome is dispensable for lytic infection, and can be replaced by unrelated DNA sequence. The limit to the size of DNA fragments which can be incorporated into a λ particle is 20 kb, which is significantly larger than fragments suitable for plasmids (around 10 kb maximum). A further advantage of λ-based vectors is that each phage particle containing recombinant DNA will infect a single cell. The infection process is about a thousand times more efficient than transformation of bacterial cells with plasmid vectors.

3.3.3 Cosmids

Both λ phage and *E. coli* plasmid vectors are useful for cloning only relatively small DNA fragments. However, several other vectors have been developed for cloning larger fragments of DNA. One common method for cloning large fragments makes use of elements of both plasmid and λ-phage cloning. In this method, called **cosmid** cloning, recombinant plasmids containing inserted fragments up to a length of 45 kb can be efficiently introduced into *E. coli* cells. A cosmid vector is produced by inserting the cos sequence from λ-phage DNA into a small *E. coli* plasmid vector about 5 kb long. Cosmid vectors contain all the essential components found in plasmids. The cosmid can incorporate foreign DNA inserts that are between 35 and 45 kb in length. Such recombinant molecules can be packaged and used to transform *E. coli*. Since the injected DNA does not encode any λ-phage proteins, no viral particles form in infected cells and likewise the cells are not killed. Rather, the injected DNA circularizes, forming in each host cell a large plasmid containing the cosmid vector and the inserted DNA fragment. Cells containing cosmid molecules can be selected using antibiotics as described for ordinary plasmid cloning.

COSMIDS
Artificial cloning plasmids, able to incorporate comparatively large DNA fragments of 35 to 45 kb.

A recently developed approach similar to cosmid cloning makes use of larger *E. coli* viruses such as bacteriophage P1. Recombinant plasmids containing DNA fragments of up to ≈100 kb can be packaged *in vitro* with the P1 system.

3.3.4 Yeast artificial chromosomes (YAC)

BACS AND YACS
Bacterial and yeast artificial chromosomes: Engineered chromosomes, that can act as vectors for very large DNA fragments.

YACs are constructed by ligating the components required for replication and segregation of natural yeast chromosomes to very large fragments of target DNA, which may be more than 1 Mb in length (Ramsay, 1994). **YAC** vectors contain two telomeric sequences (TEL), one centromere (CEN), one autonomously replicating sequence (ARS) and genes which act as selectable markers in yeast. YAC selectable markers usually do not confer resistance to antibiotic substances, as in *E. coli* plasmids, but instead enable growth of yeast on selective media lacking specific nutrients.

3.3.5 Bacterial artificial chromosomes (BAC)

BAC vectors were developed to avoid problems that were encountered with YACs to clone large genomic DNA fragments. Although YACs can accommodate very large DNA fragments they may be unstable, i.e. they often lose parts of the fragments during propagation in yeast.

In general, BACs can contain up to 300–350 kb of insert sequence. In addition, they are stably propagated and replicated in *E. coli*, are easily introduced into their host cell by transformation, large amounts can be produced in a short time due to the fast growth of *E. coli*, and they are simple to purify (Giraldo and Montoliu, 2001). The vectors are based on the naturally occurring plasmid F factor of *E. coli*, which encodes its own DNA polymerase and is maintained in the cell at a level of one or two copies. A BAC vector consists of the genes essential for replication and maintenance of the F factor, a selectable marker gene (SMG) and a cloning site for the insertion of target fragment DNA.

To summarize, cloning vectors are DNA molecules that can incorporate foreign DNA fragments and replicate in a suitable host, producing large quantities of the desired DNA fragment. Such methods are highly important for a variety of molecular biology applications and are the basis of recombinant DNA technology. However, for the production of transgenic organisms and related biotechnological applications, such vectors need to possess additional sequence elements and properties that allow targeted transfer of specific genes and controlled expression of these genes in a host organism. The necessary features to accomplish these tasks will be discussed in the following paragraphs.

3.4 PROMOTERS

As already introduced in Chapter 2, the promoter sequence is the key regulatory region of a gene that controls and regulates gene expression. More specifically, the promoter has a major importance in the regulation of transcription, i.e. the transfer of the information contained in a DNA coding region into an mRNA transcript. Promoters play an important role in the regulation of gene expression at different locations and times during the life cycle of an organism or in response to internal and external stimuli (Juven-Gershon *et al.*, 2008). Investigating and unravelling the precise function of promoter components and the additional factors associated with their performance revealed new possibilities of genetic engineering. Nowadays, it is feasible to modulate the expression of defined genes in an organism by combining them with a promoter of choice, resulting in the desired gene expression profile.

This approach can be used to modulate the expression of endogenous genes (i.e. genes that the organism possesses already) or to introduce foreign genes in combination with a foreign or endogenous promoter to create an organism with defined novel traits. Thus, promoters have a huge influence in follow-on research and development in biotechnology, and a more detailed understanding will certainly further influence the development of GMOs.

3.4.1 Types of promoters

PROMOTER TYPES
Promoters can be classified as constitutive, tissue-specific or inducible, according to their mode of regulating gene expression.

In general, promoters can be divided into different classes according to their function:

» **Constitutive promoters.** Constitutive promoters direct the expression of a gene in virtually all cells or tissues of an organism. The genes controlled by such promoters are often "housekeeping genes", i.e. genes whose products are constantly needed by the cell to survive and maintain its function. Constitutive promoters are to a large extent, or even entirely, insensitive to environmental or internal influences, thus the level of gene expression is always kept constant. Due to the insensitivity to external or internal stimuli and the high sequence conservation of such promoters between different species, constitutive promoters are in many cases active across species and even across kingdoms. An important example is the Cauliflower mosaic virus (CaMV) 35S promoter, which is frequently used to drive transgene expression in transgenic plants.

» **Tissue-specific promoters.** Tissue-specific promoters direct the expression of a gene in a specific tissue or cell type of an organism or during certain stages of development. Thus, the gene product is only found in those cells or tissues and is absent in others, where the promoter is inactive. In plants, promoter elements that specifically regulate the expression of genes in tubers, roots, vascular bundles, other vegetative organs or seeds and reproductive organs have been used for genetic engineering, both within a certain species and across different species. Frequently, such promoters rely on the presence or absence of endogenous factors to function, so in fact it is the presence or absence of these factors that defines the tissue-specificity of gene expression.

» **Inducible promoters.** Inducible promoters are of high interest to genetic engineering because their performance is dependent on certain endogenous or external factors or stimuli. In the ideal case, gene expression by an inducible

promoter can be controlled by the experimenter by simply adding a certain substance to the cell culture/the organism. This will result in expression of all genes controlled by this promoter – in the case of transgenic organisms, usually only the genes that have been specifically introduced (Padidam, 2003). Within the class of inducible promoters, one can find promoters controlled by abiotic factors such as light, oxygen level, heat, cold and wounding, while others are controlled by certain chemicals or metabolites. As it may be difficult to control some of these factors in the field, promoters that respond to chemical compounds, which are not found naturally in the organism of interest, are of particular interest. Substances that have been found to control certain promoters include rare metabolites, antibiotics, some metals, alcohol, steroids and herbicides, among other compounds. Once a promoter that responds to a certain compound has been identified it can be further engineered and adapted to induce gene expression in GMOs at will, independent of other factors encountered by the organism (Gurr and Rushton, 2005).

3.5 EXPRESSION VECTORS

Cloning a gene encoding a particular protein is only the first of many steps needed to produce a recombinant protein for agricultural, medical or industrial use. The next step is to transfer the DNA sequence containing the gene into the desired host cell for its expression and the production of the protein of interest. In order to allow expression of the gene of interest in the host cell or organism, it must be transferred into a vector that has several distinct sequence features. These features include all sequences that are required to drive and regulate expression of the gene, i.e. all components that are associated with a functional gene (see 2.8 and 2.9). Thus, in addition to the characteristics described for cloning vectors, an **expression vector** must carry a promoter, a polyadenylation site, and a transcription termination sequence. These sequences should have a correct orientation with regard to the multiple cloning site, where the foreign DNA is integrated. Inserting a coding

EXPRESSION VECTOR
A vector that, in addition to the properties of a cloning vector, contains sequences that direct expression of the inserted DNA sequence in an appropriate host organism.

sequence in proper orientation in between these expression control sequences will result in the expression of the gene in an appropriate host. A simplified version of an expression vector is depicted in Figure 3.2:

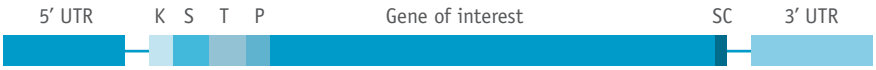
Figure 3.2 | **Generalized mammalian expression vector**

The multiple cloning site (MCS), where the foreign DNA can be inserted, and selectable marker gene (SMG) are under control of a eukaryotic promoter (p), polyadenylation (pa), and termination of transcription (TT) sequences. An intron (I) enhances the production of heterologous protein. Propagation of the vector in E. coli and mammalian cells depends on the origins of replication ori^{E} and ori^{euk}, respectively. The ampicillin gene (Amp^{r}) is used for selecting transformed E. coli cells.

In some cases, it is necessary and helpful to fuse some translation control and protein purification elements to the gene of interest (Figure 3.3) or to add them to the expression vector MCS. This is especially important if a recombinant protein is purified after its expression in a certain host cell or organism. For this purpose, short specific amino acid sequences, commonly referred to as tags, can be added to the protein by adding the sequence encoding them to the coding sequence of the protein. These tags can greatly facilitate protein purification, due to certain properties they possess and that are specific for each tag. If necessary, such tags can be removed from the final, purified protein by introducing a further specific amino acid sequence, which is recognized by a protease that cleaves the protein at this position and thus removes the tag. An example of a gene with such added sequences is given in Figure 3.3:

Figure 3.3 | **A gene of interest fitted with sequences that enhance translation and facilitate both secretion and purification of the produced protein**

These include the Kozak sequence (K) [5′-ACCAUGG-3′, its presence near the initiating AUG greatly increases the effectiveness of initiation], signal sequence (S) required for secretion, protein affinity tag (T), proteolytic cleavage site (P), and stop codon (SC). The 5′ and 3′ UTRs increase the efficiency of translation and contribute to mRNA stability.



In the case of **transgenic plant and animal** production, the general layout of an engineered gene as depicted in Figure 3.3 also holds true in most cases. However, other types of vectors to deliver the transgene to the plant or animal cells are frequently employed. Whereas cells in cell culture can be easily monitored for the presence of the desired expression vector and the expression vector is stably maintained within the cells, this is not necessarily the case for complex organisms. Therefore, the genes of interest are usually integrated in a vector that mediates integration of the transgene into the host organism's genome (i.e. into a chromosome). Thus, the transgene becomes an integral part of the organism's genome, and as such is present in all cells of an organism and is stably passed on to subsequent generations (Somers and Makarevich, 2004). This is usually not the case for plasmid vectors, which are maintained as extra-chromosomal entities and are frequently lost during cell divison and propagation.

TRANSGENIC PLANTS AND ANIMALS
Organisms, in which foreign DNA has been introduced by recombinant DNA technology.

The vectors and techniques that are employed to produce stable transgene integrations into the genome of a given organism are described in detail in the following chapters.

To summarize, this chapter has provided an introduction to the field of recombinant DNA technology. Specific DNA fragments can be cloned, by means of cloning vectors, and subsequently be isolated, investigated and further modified with great ease. Furthermore, specific DNA fragments containing protein-coding genes can be transferred to expression vectors, which will result in expression of the encoded proteins upon introduction of the vector into an appropriate host cell or organism. Thus, desired proteins can be produced in large quantities. Careful choice of the vector, the production host and promoter and other regulatory sequences is of high importance for the success of such approaches. Modern biotechnology offers the possibility to freely combine genes with promoters and other desired sequences, regardless of the original source of the genes and DNA sequences.

This technology also sets the basis for the creation of transgenic plants and animals, which are engineered to express new traits and properties by the specific introduction or modulation of genes and regulatory sequences. Plant and animal recombinant DNA techniques are introduced in the following two chapters.

CHAPTER 4

PLANT TRANSFORMATION AND SELECTION TECHNIQUES

4.1 PLANT TRANSFORMATION

In the last two chapters the molecular techniques, generally referred to as recombinant DNA technology, that allow isolation, manipulation and expression of specific genes were described. Furthermore, potential applications of this technology to produce specific proteins for medical or industrial use in cell culture were also discussed.

This chapter will provide the link between recombinant DNA technology and the creation of transgenic plants that possess novel traits of interest to agriculture, medicine or industry. This application is based on the techniques described so far, but in addition relies on novel techniques that are specific to and necessary for the creation of transgenic plants.

Genetic transformation is the (sometimes heritable) change in the genome of a cell or organism brought about by the uptake and incorporation of introduced, foreign DNA. Transformation encompasses a variety of gene transfer events, which can be characterized by the stability of transformation, the subcellular compartment transformed (nuclear, mitochondrial or plastid) and whether the transferred DNA is stably integrated into the host genome (Shewry *et al.*, 2008).

GENETIC TRANSFORMATION
The uptake of foreign DNA sequences into a cell. In some cases, this may take the form of stable incorporation of the DNA into the cell's/organism's genome.

Table 4.1 documents the generally accepted definitions of these alternative transformation events.

Table 4.1 | **Definitions of transformation**

Term	Definitions
Stable transformation	The transgene and novel genetic characteristics are stably maintained during the life of the cell culture or organism. The transgene is usually, but not necessarily, integrated into the host genome.
Transient expression	Expression of the transgene is detected in the first few days after its introduction into host cells. A subsequent decline in expression indicates that expression was based on non-integrated, extra-chromosomal DNA.
Integrative transformation	The transgene is covalently integrated into the genome of the host cell. In fertile plants (or animals) the transgene is inherited by the next generation (a form of stable integration).
Nuclear transformation	Gene transfer into the nuclear genome of the host cell, as confirmed by cellular fractionation, eukaryotic-type expression or mendelian inheritance.
Organellar transformation	Gene transfer into the plastid or mitochondrial genome of the host cell, as confirmed by cellular fractionation, prokaryotic-type expression or maternal inheritance.
Episomal transformation	Viral genomes or "mini-chromosomes" are introduced which replicate independently from the host genome. Stable over several generations in some cases.

4.2 PLANT TISSUE CULTURE

An important phenomenon that is a key determinant to plant transformation, and thus the generation of transgenic plants, is the finding that whole plants can be regenerated from single cells. Plant transformation thus depends on two events: successful introduction of foreign DNA into target plant cells, and subsequent development of a complete plant derived from the transformed cells.

PLANT REGENERATION
The possibility to regenerate complete plants from plant cell culture.

***In vitro* regeneration** is the technique of developing plant organs or plantlets from plant cells, tissues or organs isolated from the mother plant and cultivated on artificial media under laboratory conditions (Thorpe, 2007). Depending on different physical and physiological factors, in combination with various growth regulators, regeneration occurs via organogenesis (initiation of adventitious roots or shoots from plant cells or tissues) or embryogenesis (formation of plants

from somatic cells through a pathway resembling normal embryogenesis from the zygote). Both organogenesis and embryogenesis can be initiated either directly (from meristematic cells) or after formation of a callus (mass of undifferentiated parenchymatic cells induced by wounding or hormone treatment).

Transformed plants can thus be regenerated from calli or wounded plant tissues, such as leaf disks, into which foreign DNA has previously been introduced (Figure 4.1).

4.3 PLANT TRANSFORMATION TECHNIQUES

There is an expanding repertoire of **plant transformation** techniques available, ranging from established techniques to highly experimental methodologies (Newell, 2000). In Table 4.2 these alternative approaches to gene delivery are listed with brief comments on their application, efficiency and limitations. The most widely used techniques are the *Agrobacterium tumefaciens*-mediated transfer, microprojectile bombardment ("gene gun" or biolistic method) and direct gene transfer to protoplasts. The biolistic technique has proven especially useful in transforming monocotyledonous species like maize and rice, whereas transformation via *Agrobacterium* has been successfully practised in dicotyledonous species. Only recently has it also been effectively employed in monocotyledons. In general, the *Agrobacterium*-mediated method is considered preferable to the gene gun due to the higher frequency of single-site insertions of the foreign DNA into the host genome, making the transformation process easier to monitor. All available and currently employed transformation techniques are briefly described in the following sections.

PLANT TRANSFORMATION TECHNIQUES
All methods to achieve the uptake and introduction of foreign DNA into a plant's genome.

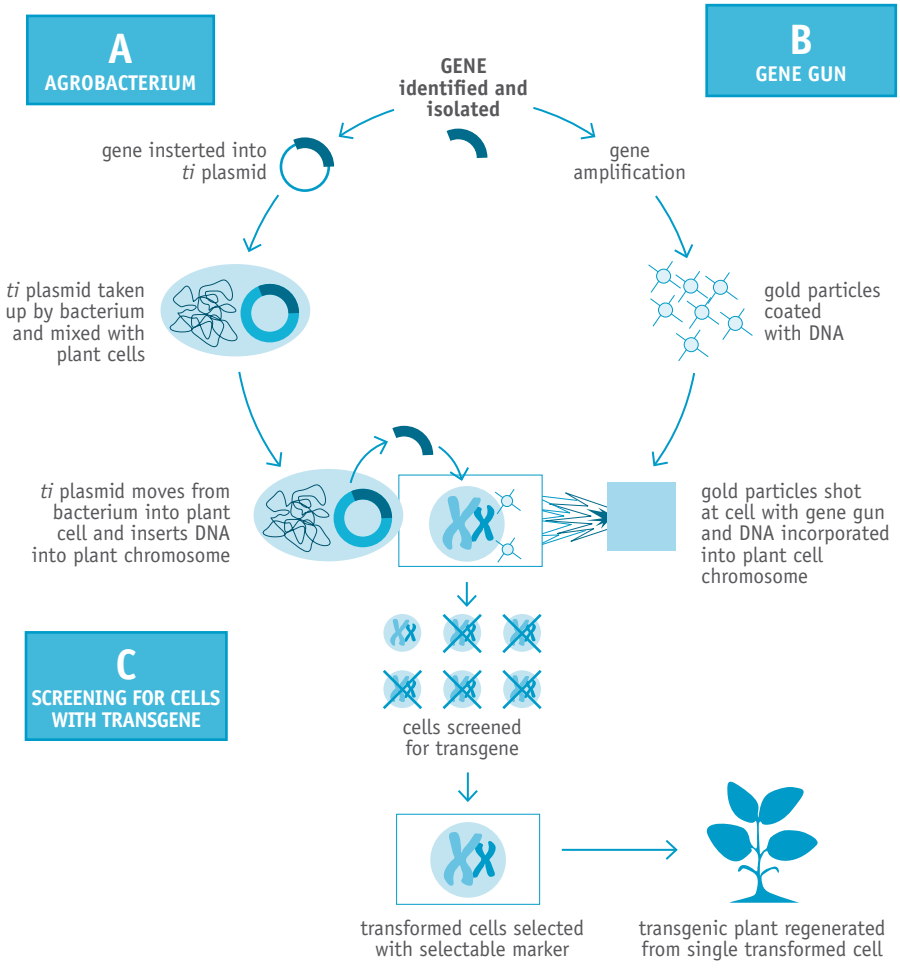
4.3.1 Microprojectile bombardment

This technique uses high velocity particles, or **microprojectiles**, that are coated with DNA and deliver exogenous genetic material into the target cell or tissue. Transformed cells are selected, cultured *in vitro* and regenerated to produce mature transformed plants (Kikkert *et al.*, 2005).

MICROPROJECTILE BOMBARDMENT
Plant cell transformation by shooting DNA-coated microparticles into plant material.

Figure 4.1 | **Steps involved in the generation of genetically transformed plants using either the *Agrobacterium tumefaciens* (*A. tumefaciens*) or microprojectile bombardment approaches**

Following introduction of foreign DNA into the plant cell, successfully transformed cells are selected and used to regenerate a transgenic plant (see text for details).



The particles, either tungsten or gold, are small (0.5-5 µm) but big enough to have the necessary mass to be sufficiently accelerated and penetrate the cell wall carrying the coated DNA on their surface. Once the foreign DNA is integrated into the plant genome in the cell nucleus, which is a somewhat spontaneous process, it can be expressed. Gold particles are chemically inert, although rather costly, and show a high uniformity. Tungsten particles, despite showing mild phytotoxicity and being more variable in size, are adequate for most studies. Furthermore, the chosen microprojectile should have good DNA binding affinity but, at the same time, be able to release the DNA once it has hit the target. DNA coating of surface-sterilized particles can be accomplished by defined DNA treatments using, for instance, the calcium chloride method, with the addition of certain chemicals to protect the DNA. However, a recent report describes the novel use of *Agrobacterium* as coating material for the microprojectiles, which are then shot into the target tissue. Once coated the particles are ready for shooting; the particles are accelerated and ultimatively collide with the target, usually plant cells or calli grown on a Petri dish. The DNA, delivered with this strategy, is expressed after reaching the nucleus and integrating randomly into the plant genome.

4.3.2 Agrobacterium-mediated plant transformation

A. tumefaciens are soil bacteria that have the ability to infect plant cells and transfer a defined sequence of their DNA to the plant cell in the infection process. Upon integration of the bacterial DNA into a plant chromosome, it directs the synthesis of several proteins, using the plant cellular machinery, that ensure the proliferation of the bacterial population within the infected plant. *Agrobacterium* infections result in crown gall disease (Gelvin, 2003).

In addition to its chromosomal genomic DNA, an ***A. tumefaciens*** cell contains a plasmid known as the Ti (tumour-inducing) plasmid. The Ti plasmid contains a series of *vir* (virulence) genes that direct the infection process, and a stretch of DNA termed

A. TUMEFACIENS
Pathogenic plant bacterium that has the ability to transfer a part of its DNA to the plant during the infection process.

T-DNA (transfer DNA), approximately 20 kb in length, that is transferred to the plant cell in the infection process. The T-DNA encodes proteins required for the maintenance of infection. These proteins include certain plant hormones that stimulate cell growth, resulting in the formation of galls, and proteins required for a certain metabolic pathway that secures the availability of nutrients for the bacteria (Figure 4.2).

Agrobacterium can only infect plants through wounds. When a plant root or stem is wounded it gives off certain chemical signals. In response to these signals, agrobacterial *vir* genes become activated and direct a series of events necessary for the transfer of the T-DNA from the Ti plasmid to the plant cell through the wound.

TI-PLASMID
The *A. tumefaciens* plasmid that is responsible for transferring DNA to the plant genome in the infection process. Engineered versions of the Ti-plasmid are used as transgene vectors for plant transformation.

To harness *A. tumefaciens* and the **Ti-plasmid** as a transgene vector, the tumor-inducing section of T-DNA is removed, while the T-DNA border regions and the *vir* genes are retained. The desired transgene is inserted between the T-DNA border regions, applying recombinant DNA technology. Thus, in the infection process, the transgene DNA is transferred to the plant cell and integrated into the plant's chromosomes (Lacroix *et al.*, 2006). To achieve transformation, *Agrobacterium* cells carrying an appropriately constituted Ti plasmid vector containing the desired transgene can be inoculated into plant stems, leaf disks etc., to allow infection and T-DNA transfer to the plant cells. The explants that have been co-cultivated with *Agrobacterium* are subsequently processed through various tissue culture steps resulting in the selection and production of transformed cells and plants.

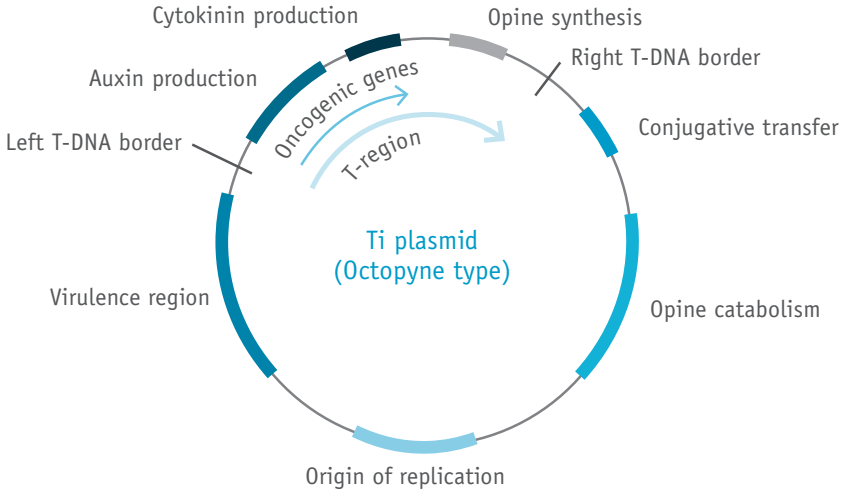
4.3.3 Protoplast transformation techniques

PROTOPLAST
Cultured plant cells whose cell wall has been removed.

One of the characteristic features of plant cells is that they are surrounded by a rigid, cellulose-based cell wall. **Protoplasts** are plant cells in which the cell wall has been removed (Davey *et al.*, 2005). Therefore protoplasts behave like animal cells, which naturally have no cell wall barrier. Plant regeneration from single protoplasts is possible due to the totipotency of plant cells, i.e. the potential of

Figure 4.2 | **Wild type Ti plasmid of *Agrobacterium tumefaciens* (*A. tumefaciens*)**

The T region, i.e. the region of the plasmid that can be exchanged and replaced with the transgene of interest, is highlighted in light blue.



a single cell to reconstitute a complete plant. Removal of the cell wall is achieved by treating the plant material (leaves, tissue cultures, suspended cells, etc.) with a cocktail of cell wall-destroying enzymes, including pectinases, cellulases, and/or hemicellulases in an appropriate incubation medium of the correct osmolality (i.e. the concentration of solutes in the medium). After removal of the cell wall, the protoplasts must be kept immersed in a solution of the appropriate solute concentration to prevent them from bursting. Thus, monitoring the correct osmolality of the culture medium until a new cell wall has formed is of high importance.

Different approaches exist for the delivery of transgene DNA into protoplasts through the plasma membrane. These include chemical treatments, electroporation and micro-injection techniques (Davey *et al.*, 2005).

4.3.3.1 Chemical techniques

The most commonly applied chemical protoplast transformation methods include polyethylene glycol (PEG) treatment, Ca^{2+}-DNA co-precipitation and liposomal DNA delivery. PEG treatment is the most widely used technique, employing solutions of 10-15 percent PEG in combination with high calcium content and a high pH. After mixing the isolated DNA and the protoplasts, followed by different washes, the DNA may be taken up by the protoplast. The role of PEG is to alter the plasma membrane properties, causing a reversible membrane permeabilization, thus enabling exogenous macromolecules to enter the cell cytoplasm.

Ca^{2+}-DNA co-precipitation depends on the formation of a co-precipitate of plasmid DNA and calcium phosphate. On contact with protoplasts under high pH conditions, the co-precipitate trespasses the cell's plasma membrane.

Liposomes, which are negatively-charged spheres of lipids, are also employed for DNA transfer and uptake into cells. DNA is first encapsulated into the liposomes which are subsequently fused with protoplasts, employing PEG as a fusogen.

4.3.3.2 Electroporation

Electrical pulses are applied to the DNA-protoplast mixture, provoking an increase in the protoplast membrane permeability to DNA. This technique is much simpler than the chemical method, providing satisfying results. However, the electrical pulses must be carefully controlled as cell death can occur above a certain threshold. The pulses induce the transient formation of micropores in the membrane lipid bilayer which persist for a few minutes, allowing DNA uptake to occur.

4.3.3.3 Micro-injection

This technique was originally designed to transform animal cells, and was later adapted for and gained importance in transforming plant cells. However, in plant cells the existence of a rigid cell wall, a natural barrier, prevents micro-injection. Furthermore, the presence of vacuoles that contain hydrolases and toxic metabolites that may lead to cell death after vacuole breakage presents a severe restriction to micro-injection. Therefore, protoplasts, rather than intact plant cells, are more suitable for micro-injection. This method is labour-intensive and requires special micro-equipment for the manipulation of host protoplasts and DNA. However, some success in transforming both monocotyledonous and dicotyledonous species has been achieved employing this technique.

4.3.4 Virus-mediated plant transformation/transduction

Virus-based vectors have been shown to be efficient tools for the transient, high-level expression of foreign proteins in plants (Chung *et al.*, 2006). These vectors are derived from plant viruses, e.g. Tobacco Mosaic Virus (TMV), and are manipulated to encode a protein of interest. Initial delivery of the virus-based vector to the plant can be achieved by *Agrobacterium* - the vector is encoded in the T-DNA, which is transferred to the plant. This method is applicable to whole plants, by the process of agroinfiltration, circumventing the need for labour-intensive tissue-culture.

Within a plant cell, the **virus-based vectors** are autonomously replicated, can spread from cell to cell and direct the synthesis of the encoded protein of interest. The advantages of this method are the applicability to whole plants and thus a much faster outcome than the establishment of a transgenic plant, and the high-level expression of the desired protein within a short time. The major disadvantage is that the process is transient: the expression level decreases over time, and the genetic change is not passed on to subsequent generations, i.e. it is not heritable.

VIRUS-BASED VECTORS
Vectors based on viral genomes, or parts thereof. Increasingly used for both stable and transient DNA transfer to cells or organisms.

The process of virus-mediated DNA transfer is referred to as transduction.

Several other plant transformation techniques, which have been reported but could not be reproduced or did not gain significant importance, are listed below in Table 4.2:

Table 4.2 | **Summary of plant transformation techniques**

Gene delivery method	Characteristics
Agrobacterium	Well-established transformation vector for many dicots and several monocots and a promising vector for gymnosperms. A wide range of disarmed Ti- or Ri-derived plasmid vectors are available. Additional value for the delivery of viral genomes to suitable hosts by agroinfiltration.
Direct DNA transfer to protoplasts	Well-established transformation technique with wide host range. Permeabilization of the plasma membrane to DNA by chemical agents or electroporation. Alternatively, genes can be delivered to protoplasts by injection or fusion with DNA in encapsulated liposomes.
Microprojectile bombardment	A widely used technique for introducing DNA via coated particles into plant cells. No host range limitation. Gene transfer to *in situ* chloroplasts has been documented.
Micro-injection	Effective gene delivery technique allowing visual DNA targeting to cell type and intracellular compartment. Labour-intensive and requiring specialist skills and equipment.
Macroinjection	Technically simple approach to deliver DNA to developing floral tissue by a hypodermic needle. Germline transformation not reproducibly reported.
Impregnation by whiskers	Suspensions of plant cells mixed with DNA and micron-sized whiskers. Both transient expression and stable transformation observed.
Laser perforations	Transient expression observed from cells targeted with a laser microbeam in DNA solution.
Impregnation of tissues	Transient and stable expression from tissue bathed in DNA solution or infiltrated under vacuum.
Floral dip	Stable DNA integration and expression following dipping of floral buds into DNA solution.
Pollen tube pathway	Claims of germ line transformation by treating pollen or carpels with DNA; remains controversial.
Ultrasonication	Stable transformation by ultrasonication of explants in the presence of DNA reported. Confirmation required.

4.4 SELECTION OF SUCCESSFULLY TRANSFORMED TISSUES

Following the transformation procedure, plant tissues are transferred to a selective medium containing a certain selective agent, depending on which SMG was used in the transgene expression cassette. Selectable markers are genes which allow the **selection** of transformed cells, or tissue explants, by enabling transformed cells to grow in the presence of a certain agent added to the medium (Miki and McHugh, 2004). One can differentiate between negative and positive selection: in positive selection, transformed cells possess a growth advantage over non-transformed cells, while in negative selection transformed cells survive whereas non-transformed cells are killed. Negative selection is the method of choice for most approaches. Thus, only cells/plants expressing the SMG will survive and it is assumed that these plants will also possess the transgene of interest. All subsequent steps in the plant regeneration process will only use the surviving cells/plants. In addition to selecting for transformants, marker genes can be used to follow the inheritance of a foreign gene in a segregating population of plants.

SELECTION
The process of selecting cells or organisms that have been successfully transformed with the desired transgene.

In some instances, transformation cassettes also include **marker/reporter genes** that encode gene products whose enzymatic activity can be easily assayed, allowing not only the detection of transformants but also an estimation of the level of foreign gene expression in the transgenic tissue. Markers such as β-glucuronidase (GUS), green fluorescent protein (GFP) and luciferase allow screening for enzymatic activity by histochemical staining or fluorimetric assays of individual cells and can be used to study cell-specific as well as developmentally regulated gene expression. These types of transgene constructs are usually used for optimizing transformation protocols and not for the development of commercial GM crops.

SELECTABLE MARKER/ REPORTER GENES
Genes that are incorporated into the transgene cassette and facilitate selection of transformed cells. Usually they confer resistance to certain antibiotic substances or allow visual detection of transformed cells.

In some cases, it may be desirable to produce a transgenic plant that does not contain the SMG used for the initial selection of transformed cells. Concerns have been raised about the release of transgenic plants containing antibiotic resistance or herbicide resistance genes, since the possibility of gene transfer to other

species cannot be ruled out. Therefore, techniques for producing marker-free plants have been developed, by either using markers not based on herbicide/antibiotic tolerance or by specifically deleting the SMG after selection of transformed cells (Darbani *et al.*, 2007).

4.5 SELECTABLE MARKER GENES (SMG)

The selectable portions on most transformation vectors are prokaryotic antibiotic resistance enzymes, which will also confer resistancy when they are expressed in plant cells. In some experiments, enzymes providing protection against specific herbicides have also been used successfully as marker genes (Miki and McHugh, 2004). The selective agent employed, i.e. the antibiotic or herbicide, must be able to exert stringent selection pressure on the plant tissue concerned, to ensure that only transformed cells survive. Below, some commonly used marker genes are briefly presented.

4.5.1 Neomycin phosphotransferase (npt-II) gene and hygromycin phosphotransferase (hpt) gene

Neomycin phosphotransferase-II (*npt*-II) is a small bacterial enzyme which catalyses the phosphorylation of a number of aminoglycoside antibiotics including neomycin and kanamycin. The reaction involves transfer of the γ-phosphate group of adenosine triphosphate (ATP) to the antibiotic molecule, which detoxifies the antibiotic by preventing its interaction with its target molecule - the ribosome. The hygromycin phosphotransferase (hpt) gene, conferring resistance to the antibiotic hygromycin, is also commonly used as selection marker.

4.5.2 Chloramphenicol acetyltransferase (CAT) gene

The chloramphenicol resistance (*cat)* gene encodes the enzyme chloramphenicol acetyltransferase (CAT) and was the first bacterial gene to be expressed in plants.

The enzyme specifically acetylates chloramphenicol antibiotics, resulting in the formation of the 1-, 3-, and 1,3-acetylated derivatives, which are inactive. Although not used as a selection system in plants, the gene is used frequently as a reporter gene in plant promoter studies.

4.5.3 Phosphinothricin acetyltransferase genes (bar and pat genes)

A commonly used herbicide is phosphinothricin (PPT, also known as Glufosinate). This compound binds to and inhibits glutamine synthethase, which is an important enzyme in the nitrogen metabolism and ammonium fixation pathways. PPT-induced glutamine synthetase inhibition results in elevated cellular ammonium levels and cell death. The enzyme phosphinothricin acetyltransferase (PAT), first identified in *Streptomyces hygroscopicus,* acetylates and thus detoxifies PPT. This allows transformed cells, or complete transgenic plants, to survive and grow in the presence of PPT.

4.5.4 β-Glucuronidase gene (GUS)

The *E. coli* β-glucoronidase gene has been adapted as a reporter gene for the transformation of plants. β-glucuronidase, encoded by the *uidA* locus, is a hydrolase that catalyses the cleavage of a wide variety of β-glucuronides, many of which are available commercially as spectrophotometric, fluorometric and histochemical substrates.

There are several features of the GUS gene which make it a useful reporter gene for plant studies. Firstly, many plants assayed to date lack detectable intrinsic glucuronidase activity, providing a null background in plants. Secondly, glucuronidase is easily, sensitively and cheaply assayed both *in vitro* and *in situ* and is sufficiently robust to withstand fixation, enabling histochemical localization in cells and tissue sections. The preferred histochemical substrate for tissue localization of GUS is 5-bromo-4chloro-3-indolyl-β-D-glucuronide (X-gluc). The advantage of

these substrates is that the indoxyl group produced upon enzymatic cleavage dimerizes to indigo which is virtually insoluble in an aqueous environment. The histochemical assay for GUS consists of soaking tissue in substrate solution and analysing the appearance of blue colour.

4.5.5 Luciferase gene

The luciferase (*luc)* gene isolated from *Photinus pyralis* (firefly) encodes the enzyme catalysing the ATP/oxygen-dependent oxidation of the substrate luciferin, resulting in the emission of light (bioluminescence). As a reporter, the gene is the basis of highly sensitive assays for promoter activity and for protein targeting sequences, involving the measurement of light emission using liquid scintillation counter photomultipliers, luminometers, X-ray film exposure or sensitive camera film.

4.5.6 Green fluorescent protein (GFP)

GFP is a widely used marker protein in modern biological research. The protein shows green fluorescence upon exposure to blue light. Originally, the protein was isolated from the jellyfish *Aequorea victoria*, but nowadays several other varieties from other marine organisms, as well as engineered versions (with different colour fluorescence), are available. GFP is widely applied for studies addressing gene expression or promoter efficiency as well as protein localization, stability and degradation.

4.6 MOLECULAR ANALYSIS OF TRANSGENIC PLANTS

After the successful transformation and selection of plant cells and the subsequent regeneration of a transgenic plant (see 4.2), it is desirable to monitor the presence of the transgene in the plant and to investigate the expression levels of the introduced genes encoding the protein(s) of interest (Stewart, 2005).

Analysis of transgenic plants at the molecular level is mainly performed by PCR (Box 7.1) and Southern blot analysis. PCR indicates the presence of the desired transgene within the plant, whereas stable integration of the transgene into the cellular genome is confirmed by Southern blot analysis. If plants are analysed that have been transformed using *A. tumefaciens*, it is important to prepare plant DNA from sterile tissue, as contamination with *A. tumefaciens* DNA will interfere with the interpretation of the results. Southern blot analysis using genomic DNA also yields information on the copy number of the integrated DNA sequences, whether any multiple inserts are tandemly linked or dispersed throughout the genome, and on the stability of the integrated DNA in the F_1 progeny of the transformed plants.

ANALYSIS OF TRANSGENIC PLANTS
To verify the presence of the desired transgene and the expression level, i.e. the accumulation of the desired protein(s).

Molecular analysis of the protein expression levels, including tissue-specific expression, developmental stage-specific expression, expression upon certain stimuli and so on, can be assayed by enzyme-linked immunosorbent assay ELISA (Box 7.2) or immunostaining of plant tissue. Expression of a gene of interest can also be assayed by determining the presence and quantity of the corresponding RNA transcript, e.g. by applying a modified PCR protocol (reverse transcriptase PCR) [RT-PCR]). All techniques will be described in detail in Chapter 7.

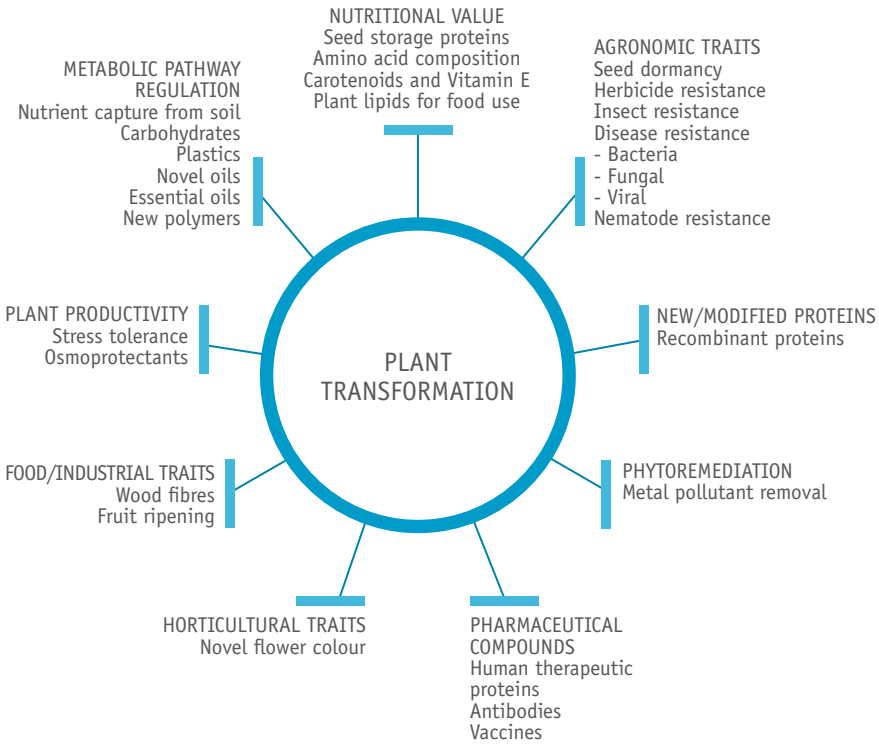
4.7 APPLICATION OF TRANSGENIC PLANTS

Numerous applications of transgenic plants are already reality or are envisaged and under investigation for the future; the main transgene targets being pest resistance and herbicide tolerance. In addition, resistances to abiotic stresses, such as drought, or improved nutrient profiles are increasingly investigated. Further possible applications that are under development are the production of medically valuable proteins or chemicals in plants (biopharmaceuticals), or the production of edible plants containing vaccines. In recent years, the technique of gene stacking, i.e. the introduction and targeting of several traits within one plant species, has also gained significant importance. Since the applications of transgenic plants are

PEST RESISTANCE AND HERBICIDE TOLERANCE
To date, these traits are the main targets for the development of transgenic plants.

diverse and numerous, no complete coverage of the field will be provided at this point. Please refer to the Annex for selected examples of transgenic plant applications explained in more detail and containing relevant literature references.

Figure 4.3 | **Applications of transgenic plants that are already available or envisaged for the future**



Adapted from: Newell, 2000.

CHAPTER 5

BIOTECHNOLOGY IN ANIMAL PRODUCTION

Modern biotechnology provides a number of possible applications in animal and livestock production. Research and development in the field focuses on improving animal growth, enhancing reproduction rates, enhancing breeding capacity and outcomes, improving animal health and developing new animal products (Basrur and King, 2005). The major techniques used to achieve these goals are the creation of transgenic animals, manipulation of animal reproduction, marker-assisted selection (MAS), molecular disease diagnostic and application of biotechnology to modify animal feed.

Once again, these technologies and applications are to a large extent based on the principles described in Chapters 2 and 3. This chapter looks at these biotechnological applications in detail and how they are impacting on animal improvement and production. Important to note is that to date no genetically modified agricultural animal has been approved for commercial release, in sharp contrast to the numerous commercial applications of GM plants. However, other biotechnological applications, which are not based on GMO production, are successfully applied in the field of animal and livestock industry.

5.1 BIOTECHNOLOGY IN ANIMAL BREEDING AND REPRODUCTION

Animal breeding, nowadays, is a field that is influenced by a whole range of biotechnological applications and developments (Bazer and Spencer, 2005). The common goal of all efforts undertaken in this field is **genetic progress** within a population, i.e. the improvement of the genetic resources and, ultimately, the phenotypic outcome. Genetic progress is influenced by several factors, namely the accuracy of choosing candidates for breeding, the additive genetic variation within the population, the selection intensity (i.e. the proportion of the population selected for further breeding), and the generation interval (the age of breeding). Note that the first three factors need to be increased in order to increase genetic progress, whereas the last factor, being generation interval, needs to be decreased. All factors can be influenced, to a varying extent, by modern biotechnology.

GENETIC PROGRESS
The improvement of the genetic resources within a population.

The techniques that are currently available to reach this end can be divided into two different groups. The first group includes all technologies that interfere with reproduction efficiency: artificial insemination, embryo transfer (ET), embryo sexing, multiple ovulation, ova pick-up and cloning, amongst others. The outcome of these technologies is an increased breeding accuracy, selection intensity and, in some cases, a shortened generation interval.

The second group of applications is based on the molecular determination of genetic variability and the identification of genetically valuable traits and characteristics. This includes the identification and characterization of **quantitative trait loci** (QTL) and the use of molecular markers for improved selection procedures. Quantitative traits are phenotypic characteristics that show a distribution of expression degree within a population (usually represented by a normal distribution), and that are based on the interaction of at least two genes (also known as polygenic inheritance). A typical example in humans is skin colour, which is based on the interaction of several genes

QUANTITATIVE TRAIT LOCI
Genes, or associated sequences, that contribute to a trait that is based on the influence of many different genes.

resulting in a large variety of phenotypes. A QTL is a DNA sequence that is associated with a certain quantitative trait – not even necessarily a gene that contributes to the trait, but possibly a sequence that is close in space to involved gene(s). Knowledge of the loci responsible for a certain quantitative trait and the underlying genes can help to select individuals for further breeding, or to start genetic engineering of the trait in question. Below, the most frequently applied techniques in animal breeding and reproduction will be summarized and explained in more detail.

5.1.1 Artificial insemination (AI)

ARTIFICIAL INSEMINATION Collection of semen from a particular individual and using it for the insemination of several female animals.

A**rtificial insemination** (AI) is the process of collecting semen from a particular male (e.g. a bull) that is subsequently used for the fertilization of many females (e.g. cows) (Galli *et al.*, 2003). The semen can be diluted and preserved by freezing (cryopreservation). This technique can enable a single bull to be used for fertilization simultaneously in several countries for up to 100 000 inseminations a year. The high intensity and accuracy of selection arising from AI can lead to a four-fold increase in the rate of genetic improvement in dairy cattle relative to that from natural mating. Since its establishment in the 1950s, AI has proven to be a very successful biotechnology, greatly enhancing the efficiency of breeding programmes (Rege, 1994). Use of AI can reduce the transmission of venereal diseases in a population and the need for farmers to maintain their own breeding males. Furthermore, it facilitates more accurate recording of pedigree and minimizes the cost of introducing improved stock. AI has significant importance for the breeding of cattle, swine and poultry.

5.1.2 Embryo transfer (ET)

EMBRYO TRANSFER Recovery or *in vitro* production of embryos and subsequent transfer to a foster mother.

Although not economically feasible for commercial use on small farms at present, **embryo technology** can greatly contribute to research and genetic improvement in local breeds. There are two procedures presently available for the production of embryos from donor females (McEvoy *et al.*, 2006). One consists of superovulation

using a range of hormone implants and treatments, followed by AI and then flushing of the uterus to gather the embryos. The other, called *in vitro* fertilization (IVF) consists of recovery of eggs from the ovaries with the aid of the ultrasound-guided transvaginal oocyte pick-up (OPU) technique. When heifers reach puberty at 11-12 months of age, their oocytes may be retrieved weekly or even twice a week. These are matured and fertilized *in vitro* and kept until they are ready for implantation into foster females. In this way, high-value female calves can be used for breeding long before they reach their normal breeding age. IVF facilitates recovery of a large number of embryos from a single female at a reduced cost, thus making ET techniques economically feasible on a large scale. Additionally, IVF produces embryos suitable for cloning experiments. However, ET is still not widely used despite its potential benefits.

5.1.3 Embryo sexing

Technologies for rapid and reliable sexing of embryos allow the generation of the desired sex at specific points in a genetic improvement programme, markedly reducing the number of animals required and enabling increased breeding progress. A number of approaches to the sexing of semen have been attempted; however, the only method of semen sexing that has shown any promise has been the sorting of spermatozoa according to the DNA content by means of flow cytometry (Rath and Johnson, 2008). Embryo sexing has been attempted by a variety of methods, including cytogenetic analysis, assays for X-linked enzyme activity, analysis of differential development rates, detection of male-specific antigens, and the use of Y-chromosome specific DNA sequences.

ANIMAL CLONING
The process of producing organisms that are genetically identical.

5.1.4 Animal cloning

A**nimal cloning** is defined as the process of producing organisms that are genetically identical. The cloning of animals can be achieved by two strategies: embryo splitting

and somatic cell nuclear transfer (SCNT) (somatic cell cloning). Both techniques offer the possibility for creating clone families from selected superior genotypes and to produce commercial clone lines (Vajta and Gjerris, 2006).

Somatic cell cloning is based on the procedure of removing the DNA from an unfertilized oocyte and replacing it with the DNA obtained from a somatic cell. The somatic cell DNA can be obtained from any individual, preferably an individual with desirable traits. Once introduced to the oocyte, the somatic cell's DNA is reprogrammed by the oocyte and the unfertilized oocyte can develop as an embryo. The resulting animal will be genetically identical as the somatic cell donor. In theory it is possible to obtain practically unlimited numbers of somatic donor cells from an individual, which allows cloning technology to be applied for the production of many genetically identical individuals. In addition, this technique offers another advantage: the somatic cells genome can be subjected to genetic manipulation prior to the introduction into the oocyte, resulting in a transgenic organism (see 5.3.3).

Embryo splitting, the second cloning technology, is the process of dividing a developing embryo, typically at the 8-cell stage, into two equal parts that continue to develop. The procedure can be repeated several times, but usually only four viable embryos can be obtained from a founder embryo. The technology has no significant importance in research and development nowadays.

5.2 GENETIC MARKERS AND MARKER-ASSISTED SELECTION (MAS)

A genetic marker is defined as a DNA sequence that is associated with a particular trait, in terms of spatial proximity of sequence, and thus segregates in an almost identical and predictable pattern as the trait. This marker can include the gene (or a part thereof) which is responsible for the trait, or DNA sequences that are sufficiently close to the gene(s) so that co-segregation is ensured.

GENETIC MARKER
A gene, or a sequence that is closely associated with it, that is responsible for a particular trait and that can be used to follow the inheritance of that trait within a population.

Genetic markers facilitate the "tagging" of individual genes or small chromosome segments containing genes which influence the trait of interest. Availability of large numbers of such markers has raised the likelihood of detection of major genes influencing quantitative traits. The process of selection for a particular trait using genetic markers is called marker assisted selection (MAS). MAS can accelerate the rate of breeding progress by increasing the accuracy of selection and by reducing the generation interval. Marker identification and use should enhance future prospects for breeding for such traits as tolerance or resistance to environmental stresses, including diseases (Dekkers, 2004; FAO, 2007).

Two types of marker can be considered. First, markers that are sufficiently close to the trait gene on the chromosome so that, in most cases, alleles of the marker and the trait gene are inherited together. This type of marker is called a linked marker. At the population level, alleles at linked markers cannot be used to predict the phenotype until the association between alleles at the marker and alleles at the trait gene is known (called "phase"). To determine phase, inheritance of the marker and trait gene has to be studied in a family. However, information on phase is only valid within that family and may change in subsequent generations through recombination (Ron and Weller, 2007).

The second type of marker is a functional trait. These markers are called "direct" markers. Once the functional polymorphism is known it is possible to predict the effect of particular alleles in all animals in a population, without first having to determine the phase. Therefore, "direct" markers are more useful than "linked" markers for predicting the phenotypic variation of target traits within a population. A further complication is that the mechanisms of genetic control differ between traits. The variation seen in some traits is directly controlled by a single gene (monogenic traits), which may have a limited number of alleles. In the simplest situation a gene will have two alleles: one allele will be associated with one phenotype and another allele with a different phenotype. An example is black

versus brown coat colour in cattle: the brown coat colour occurs as a result of a mutation in the melanocyte hormone receptor gene, which results in the creation of a different allele with a different function.

However, the traits that are important in livestock production are generally more complex and have a very large range of variation in the observed phenotype, caused by the interaction of multiple genes (polygenic traits). Growth rate and milk yield are examples of two traits that exhibit a continuous phenotypic variation. Such traits are called quantitative traits. The variation in quantitative traits is controlled by several genetic loci (called quantitative trait loci [QTL]), each of which is responsible for a small amount of the overall variation (Rocha *et al.*, 2002). The behaviour of genes (including major genes) that control a trait is likely to be dependent on the genetic background.

The myostatin allele responsible for double muscling in Belgian Blue cattle is also found in other breeds; however, the phenotype associated with the allele is variable between the breeds. This suggests that there are genes at other loci in the genome that act to modify the phenotypic expression of the major gene. Thus, information is required not only on the major genes that control a trait, but also on the interactions between genes. It is therefore premature to start using DNA-based selection widely, without further knowledge of gene interaction networks. However, some DNA tests for specific polymorphisms are being offered commercially, e.g. the GeneSTAR test for tenderness (based on variations in the calpastation gene, Pfizer Animal Genetics) and marbling (based on variations in the thyfoglobulin gene), and the Igenity test for fat deposition (based on variations in the leptin gene, Merial). These tests can be used by breeders and evaluated in their populations.

MAS and gene mapping are also considered as important tools to investigate, maintain and conserve the genetic diversity and the genetic resources of agricultural species. During the last decades an increasing portion of breeds became extinct, mainly local breeds that are not used in a sustainable manner and are not covered by breeding programmes. However, these local breeds are of high importance since they are adapted to local conditions, contribute to local food security and represent a unique source of genes that can be used for the improvement of industrial breeds. Molecular marker techniques can play an important role in the characterization and protection of agricultural genetic resources (FAO, 2006).

5.3 TRANSGENIC ANIMALS

TRANSGENIC ANIMAL
An animal, into which foreign DNA has been introduced.

A transgenic animal is an animal that carries a specific and deliberate modification of its genome – analogous to a transgenic plant. To establish a **transgenic animal**, foreign DNA constructs need to be introduced into the animal's genome, using recombinant DNA technology, so that the construct is stably maintained, expressed and passed on to subsequent generations. The last point, heritability of the genetic modification, can be achieved by creating an animal that carries the modification in the genome of its germ line: all offspring derived from this animal will be completely transgenic, as they will carry this modification in all their somatic and germ line cells.

Transgenic animals can be created for a variety of different purposes: to gain knowledge of gene function and further decipher the genetic code, study gene control in complex organisms, build genetic disease models, improve animal production traits, and produce new animal products (Melo *et al.*, 2007). This chapter will focus on the last two points, which are most important with respect to agricultural applications.

In 1982, the first transgenic animal was produced: a mouse, obtained by micro-injection of a DNA construct into a fertilized, single-cell stage oocyte (Palmiter *et*

al., 1982). The transgene construct used was composed of the rat growth hormone gene, fused to the mouse metallothionein-I promoter. The study was published in *Nature* magazine, and the impressive outcome of the study was chosen as the cover photo: the produced transgenic mice were unnaturally large, approximately twice the size as non-transgenic control mice. The impact of this study on both the scientific and public community was huge, and raised speculations about the potential applications of this technology for animals of agricultural importance. Since the insertion of a single growth hormone gene was sufficient to have tremendous effects on mice, it was anticipated that this procedure would also be applicable for agricultural animals, resulting in highly increased growth rate, feed efficiency and reduced fat deposition. Many other possible applications were also subject of speculation, such as a manipulation of milk production or production of milk with novel ingredients, increased wool production or increased resistance of farm animals to diseases and parasites.

By 1985, transgenic pigs and sheep had been obtained, with cattle and chicken following somewhat later (Melo *et al.*, 2007). Since that time the development of transgenic animals and the exploration of agricultural applications has been a steady process, although at a slower rate than what was initially expected. Engineering a specific trait proved to be much more difficult than simply introducing the responsible gene, and technical limitations, the high costs of the process and insufficient knowledge about gene function and regulation of gene expression severely restricted progress. This is particularly true for agricultural species such as cattle, which proved to be much more complicated than mice.

Nevertheless, research in the field continues and several agricultural applications are envisaged, and the approval and market release of the first transgenic animals is expected to take place in the next few years. The knowledge of gene expression and regulation is constantly extending, facilitating genetic engineering in complex animals, such as mammals. Likewise, the repertoire of available techniques to

manipulate DNA and animals is constantly increasing. Therefore, it is likely that genetic engineering techniques applied to animals of agricultural importance will play an increasingly important role in the years to come. The techniques that are currently applied to produce transgenic animals are listed below.

5.3.1 Micro-injection

MICRO-INJECTION
The first approach developed for the production of transgenic animals, based on the injection of the transgene into fertilized oocytes.

Micro-injection, the first successful approach for the creation of transgenic animals, has already been described in the preceding paragraphs. Briefly, it is based on the injection of a foreign DNA construct into a fertilized oocyte (Figure 5.1). The construct integrates randomly into the host oocyte genome, subsequently the zygote continues embryonic development, the embryo is transferred to a foster mother and eventually develops to a transgenic animal. However, this method has strong limitations: on average, less than 1 percent of embryos injected and 10 percent of animals born are transgenic, genes can only be added, not replaced or deleted, and multiple copies of the transgene are inserted at random, hindering the correct regulation of gene expression and possibly interfering with endogenous gene function (Robl *et al.*, 2007). This requires large amounts of oocytes to be injected, as the overall efficiency of the process is very low and basically a trial-and-error process, whose outcome can only be influenced to a small extent.

5.3.2 ES cell based cloning and transgenesis

EMBRYONIC STEM CELLS
Cells, derived from an early embryo, that possess the capability to differentiate into any of the cells of the adult animal (pluripotency).

To overcome the problems associated with micro-injection techniques, **embryonic stem cell** (ES cell) technology has been developed (Denning and Priddle, 2003). Embryonic stem cells, as the name suggests, are derived from embryos at a very early stage (the blastula), and possess the important characteristic of pluripotency. Pluripotency is the ability of these cells to differentiate to any of the cell types and tissues found in the adult organism. ES cells can be grown in culture for many passages and can be subjected to transformation with transgene constructs, resulting in modifications

of their genome. The constructs used not only permit the selection of successfully transformed cells, but also allow **gene targeting** to be accomplished (see 5.3.3) Thus, genes can be specifically introduced, replaced or deleted (so-called knock-ins and knock-outs). Transformed ES cells are re-introduced into the blastocoel cavity of an embryo, where they integrate and produce a mosaic (chimaeric) animal, i.e. an animal that is made up of transformed and non-transformed cells. Possibly, the chimaeric animal carries the transgene in the germ line; in this case, it is possible to obtain completely homozygous transgenic animals through selective breeding (Figure 5.1).

GENE TARGETING
The possibility to use gene constructs that integrate or replace DNA sequences at specific, determined sites in the host genome. Usually performed with ES cells.

This technique, mainly through the feature of gene targeting, allows a broad variety of genetic modifications to be introduced. For many years, several laboratories worldwide have tried to produce ES cells from farm animals, and although some success has been claimed, no robust and reproducible method has been published. Indeed, even in mice the production of ES cells is a costly and labour-intensive technology (Melo *et al.*, 2007).

5.3.3 Somatic cell nuclear transfer (SCNT)

The method of choice nowadays for the production of transgenic animals is **somatic cell nuclear transfer (SCNT)**. This method, also known as somatic cell cloning, initially gained importance for the possibility to clone animals in theoretically unlimited numbers (see 5.2.4). However, it can also be adapted to produce transgenic animals, with the additional benefit of targeted genetic manipulation (Heyman, 2005).

SOMATIC CELL NUCLEAR TRANSFER
A certain cloning procedure, based on the removal of a nucleus from a body cell of an animal and its introduction to a enucleated zygote, which subsequently develops into an animal with the identic genome as the donor animal.

The insertion of a transgene construct into a specific, pre-determined DNA site of the host genome is called gene targeting. The process and the construction of the transgene is more complex than random gene insertion, as is the case during micro-injection. Nevertheless, gene targeting is a powerful and widely used technique due to the ability to insert the transgene into a specific site (knock-in), inactivate

specific genes (knock-out) or replace the endogenous version of a gene with a modified version. This helps to overcome many of the problems and limitations that are associated with random transgene insertion.

The usual procedure is to produce a series of transgenic founder animals of both sexes, which are subjected to breeding, with the aim of producing homozygous offspring. The entire method is based on the following protocol: oocytes from a donor animal are enucleated, i.e. their nucleus containing the genome is removed. Subsequently a donor nucleus is injected into the enucleated oocyte, and the cells are fused by electrofusion. Following fusion, the oocyte is activated by chemical or mechanical means to initiate embryonic development, and the resulting embryo is transferred to a foster mother (Hodges and Stice, 2003). The donor nuclei can be derived from either somatic cells or ES cells that have been subjected to targeted genetic manipulation prior to injection into the oocyst. Thus, a large number of identical animals with targeted genetic modifications can be obtained (Figure 5.1).

Homozygous transgenic animals may also be obtained by a slightly modified approach: by targeting a transgene to one member of a pair of chromosomes, and subsequently target the same site on the other chromosome with the same transgene (Robl *et al.*, 2007).

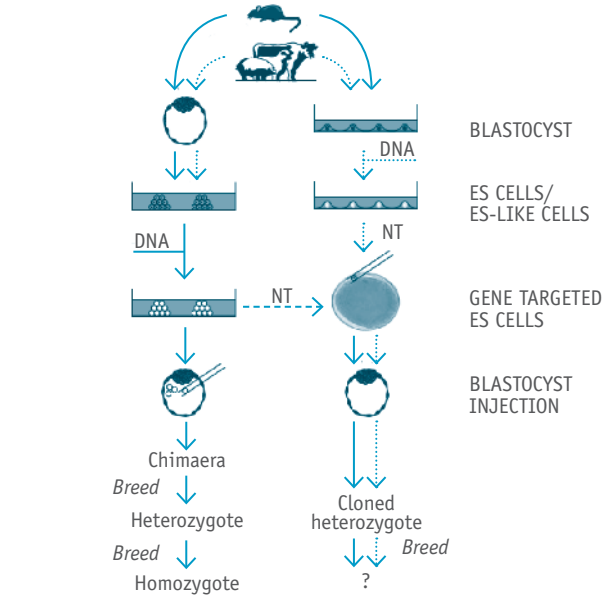
Another approach based on the techniques outlined above makes use of a rejuvenation system for bovine fibroblast (connective tissue) cells (Kuroiwa *et al.*, 2004). A bovine fibroblast cell line is derived from a bovine foetus, and subjected to genetic manipulation. Such primary cell lines grow for only a limited number of cell divisions in culture, allowing only a limited number of genetic manipulations to be introduced (usually only one) before the cells stop dividing and eventually die (Robl *et al.*, 2007). After the genetic manipulation, the cells are used in a cloning procedure to obtain cloned foetuses. These foetuses can be subjected to

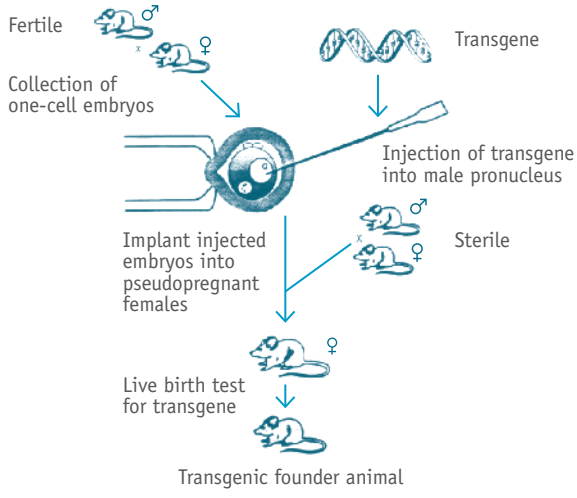
Figure 5.1 | **Comparison of micro-injection, ES cell techniques and somatic cell nuclear transfer (SCNT) for the creation of transgenic animals**

(A) ES cells are obtained from an early embryo, can be subjected to genetic modification in culture and are subsequently re-injected into an embryo or are used for nuclear transfer (NT) to an enucleated oocyte. Transgenic animals can be obtained by breeding in both cases.

(B) In SCNT, the nucleus from a somatic donor cell is removed and injected into an enucleated oocyte, resulting in a cloned animal.

(C) During micro-injection, the DNA construct is injected into a fertilized oocyte, resulting in random DNA integration and the production of a transgenic founder animal.





a second round of fibroblast isolation, manipulation and cloning. Once the genetic manipulations are completed, the final cell line can be used in a cloning procedure to produce transgenic offspring.

5.3.4 Artificial chromosome transfer

ARTIFICIAL CHROMOSOMES Chromosomes, designed by recombinant DNA technology, that can be used to transfer large pieces of foreign DNA to target organisms, where they are stably maintained and expressed.

Artificial chromosomes are a relatively recent development in animal transgenics (Robl *et al.*, 2003). One outstanding characteristic is their ability to carry very large fragments of DNA, up to several Mb (compared with 5-30 kb on a typical plasmid vector). **Artificial chromosomes** possess a centromere, telomeres and origins of replication, sequences that are responsible for their stable maintenance within the cell as autonomous, self-replicating chromosomes. This eliminates the need for integration into the host genome. Due to these properties, artificial chromosomes can be used to transfer either very large, complex genes or many small genes and regulatory elements to a target animal. The actual process of chromosome transfer and subsequent cloning of animals is similar to the SCNT approach.

The feasibility of this technique has been proven by the transfer of a human artificial chromosome, encoding the human antibody genes of 10 Mb in size to cattle (Kuroiwa *et al.*, 2002). The transferred chromosome was stable in the adult transgenic animals, and the encoded antibody genes were expressed to a certain extent.

5.3.5 Sperm-mediated DNA transfer

Several reports describe the use of sperm as a vector to deliver transgene DNA to the oocyte during the process of fertilization. The efficiency of this process varies considerably between species, and several approaches are under investigation to improve uptake and incorporation of foreign DNA. These include, among others, intracytoplasmic injection of DNA-coated sperm into the oocyte, or liposome

treatment of sperm to facilitate DNA uptake (Robl *et al.*, 2007). Nevertheless, the entire process is not completely understood and far from being used routinely.

5.3.6 Viral-vector mediated DNA transfer

Transgenesis may also be accomplished by employing virus-derived vectors, namely vectors based on the retrovirus-class of lentiviruses (Whitelaw *et al.*, 2008). Genes that are essential for viral replication are deleted from the viral genome, maintaining only the capacity for integration of the viral genome into the host genome. Parts of the vector that were occupied by viral genes can then be replaced by the transgene of interest – an approach analogous to the modification of the *A. tumefaciens* Ti-plasmid. Viruses carrying the modified vector are then produced *in vitro* and subsequently injected into the perivitelline space of the zygote (or an unfertilized oocyte), resulting in infection of the zygote and integration of the viral genome into the host genome. Transgenesis rates reaching up to 100 percent of injected embryos have been described (Park, 2007).

Major drawbacks of this method are a limited transgene size and random transgene integration. The maximal transgene size is 8 kb, which is rather low compared with other techniques. Random and possibly multiple transgene integration may lead to position effects, disturbance of the host genome and dose effects, as is the case with pronuclear injection. Solving these problems holds great promise for the further development and application of lentiviral vectors.

Other, less frequently used, methods include biolistics, liposome-mediated DNA transfer to cells and embryos, or DNA transfer to cells and embryos by electroporation. As mentioned in the introduction, constant progress is being made in the field of animal transgenesis, although no approval for commercial release has been obtained so far. Some of the envisaged applications of GM animals are given in the following paragraph.

5.4 APPLICATIONS FOR TRANSGENIC ANIMALS

Since the production of the first transgenic mice almost three decades ago much work has been performed on the development of technologies for efficient transgenesis. Many initial problems, such as low efficiencies, random transgene insertions and unexpected and undesirable behaviour of transgenic animals, have been overcome or are at least understood in more detail. Furthermore, an ever-increasing knowledge of genes, gene function and regulation of gene expression facilitates the planning and creation of transgenic animals with desired traits.

The main interest of modern agricultural research with regard to transgenic animals can be divided into two broad categories: production of animals with improved intrinsic traits, such as higher growth rates, improved milk production, disease resistance etc. The other is the production of animals that produce novel products, such as pharmaceuticals, proteins of medical relevance, vaccines etc. (Wheeler *et al.*, 2003; Niemann *et al.*, 2005). Examples of both categories will be given in the following sections.

5.4.1 Transgenic animals for food production

Engineering transgenic animals with an application in food production focuses mainly on improved meat production, improved carcass quality and enhanced milk production.

Milk is a complex biological fluid and has a high importance for contributing to the nutrition of many societies (Melo *et al.*, 2007). The major goals for transgenic animal development concerning milk production are increased milk production, higher nutrient content or milk containing novel substances.

Most milk proteins (circa 80 percent) belong to the caseins, and transgenic cattle were created that contain extra copies of casein genes. This resulted in elevated casein protein levels in milk (Brophy *et al.* 2003). Another milk application that is being investigated is the production of milk with no lactose (milk sugar) present,

since approximately 70 percent of the world population cannot metabolize lactose and thus cannot consume dairy products. Engineering milk with novel properties, e.g. milk containing the immune-stimulating human protein lactoferrin, is a further approach. Many other additives, e.g. different growth hormones or substances that stimulate health and development, have been proposed for overexpression in milk and thus possibly contribute to growth and health of developing offspring. In pigs, the transfer of the bovine a-lactalbumin gene led to increased milk production, resulting in faster piglet growth and survival rate.

One of the first reports with relevance for enhanced meat production was the article about the first transgenic mice, expressing rat growth hormone and showing increased body size and mass. However, transferring this approach to pigs initially did not yield promising results. Nevertheless, pigs showing increased muscle weight gain and feed efficiency by introducing porcine growth hormone or human insulin-like growth factor have been created (Niemann *et al.*, 2005). Furthermore, pigs expressing the enzyme phytase in their salivary glands have been created: these animals can metabolise the phosphor present as phytic acid in corn and soy products, thus needing less phosphor as feed additives and releasing less phosphor with their manure, reducing the environmental impact of pig farming (Haefner *et al.*, 2005).

Experiments in cattle are focusing on the myostatin gene, a negative regulator of muscle mass, resulting in a high increase in muscle mass in animals with a myostatin mutation or deletion.

Transgenesis is also employed for fish; injection of embryos with constructs containing either the bovine or Chinook salmon growth hormone has been reported, with the aim of improving fish growth in general and especially under adverse conditions, e.g. low water temperatures. This has resulted in an up to 5-11 fold increase in weight after one year of growth for transgenic salmon and 30-40 percent increased growth of transgenic catfish (Wheeler, 2007).

All these studies demonstrate the fundamental feasibility of applying transgenesis to agricultural animals for improved food production, but so far no transgenic food producing animal has been released for commercial use. In addition to the research and development necessary for the establishment of a transgenic animal, there are several other factors that strongly influence the use of transgenic animals for food production. Among these are considerations concerning the economic practicability, social acceptance of transgenic food and, possibly most important, regulations concerning the approval of GMOs and derived products.

Regulatory authorities need to consider three factors:

- safety of the food product for human consumption;
- environmental impact of the genetically modified animals;
- welfare of the animals.

These factors need to be considered on a case-to-case approach for every new transgenic animal or product that has been obtained using GMOs. In principle, this safety investigation is identical to the safety regulations and procedures that apply for transgenic plants. A detailed description of the safety evaluation procedures and the underlying regulatory documents and treaties is provided in the accompanying modules of this compendium.

5.4.2 Transgenic animals for production of human therapeutics

One major application of animal transgenesis nowadays is the production of pharmaceutical products, also known as animal pharming. The costs for producing transgenic animals are high, but since the pharmaceutical industry is a billion-dollar market the input is likely to be a feasible and economically worthwhile investment (Sullivan *et al.*, 2008). Since many human proteins cannot be produced in micro-organisms and production in cell culture is often labour-intensive with low yields, the

production of **biopharmaceuticals** in transgenic animal bioreactors is an attractive alternative (Kind and Schnieke, 2008). Furthermore, many human proteins cannot be produced in micro-organisms, since they lack post-translational modification mechanisms that are essential for the correct function of many human proteins.

BIOPHARMACEUTICAL Pharmaceuticals, produced in transgenic organisms (bioreactors) or in cell culture.

Pharmaceutical proteins or other compounds can be produced in a variety of body fluids, including milk, urine, blood, saliva, chicken egg white and seminal fluid, depending on the use of tissue-specific promoters (Houdebine, 2009). Nevertheless, milk is the preferred medium due to its large production volume. Furthermore, it has been shown that the mammary glands can produce up to 2 g of recombinant protein per litre of milk; assuming average protein expression and purification levels, only relatively small herds of transgenic animals would be required to supply the world market with a specific recombinant protein (e.g. 100 transgenic goats for the production of 100 kg monoclonal antibodies required per year [Melo *et al.*, 2007]). In Table 5.1, biomolecules expressed in mammary glands and their anticipated applications are listed:

Table 5.1 | **Pharmaceuticals produced by transgenic animals**

Pharmaceutical	Bioreactorspecies	Application/treatment	Company
Antithrombin III	goat	thrombosis, pulmonary embolism	GTC Biotherapeutics (USA)
tPA	goat	thrombosis	PPL Therapeutics (UK)
α-antitrypsin	sheep	emphysema and cirrhosis	PPL Therapeutics (UK)
Factor IX	sheep	hemophilia b	PPL Therapeutics (UK)
Factor VIII	sheep	hemophilia a	PPL Therapeutics (UK)
Polyclonal antibodies	cattle	vaccines	Hematech (USA)
Lactoferrin	cattle	bactericide	Pharming Group (NED)
C1 inhibitor	rabbit	hereditary angioedema	Pharming Group (NED)
Calcitonin	rabbit	osteoporosis and hypercalcemia	PPL Therapeutics (UK)

Adapted from: Melo *et al.*, 2007.

Another advantage of biopharmaceutical production in transgenic animals is the reduced risk of transmitting diseases, compared with human-derived material. Several cases are known where hundreds of patients were infected with HIV, Hepatitis C or Creutzfeld-Jakob-Disease following treatment with human-derived pharmaceuticals. Of course, animal-derived material needs to be subjected to a thorough purification procedure to exclude transmission of animal diseases (zoonoses) or contamination with animal DNA or protein that might induce an immune reaction.

Nevertheless, the development of transgenic animals that secrete high contents of the desired product in their milk, and the subsequent development of an effective and high-yield purification protocol to get rid of contaminating proteins, requires a lot of knowledge and financial and intellectual input. So far, only GTC Biotherapeutics Antithrombin III has been approved for the United States market and is sold under the name of ATryn (FDA, 2009). Furthermore, many potential target proteins as well as the technologies to develop a transgenic animal are covered by patents and intellectual property rights, thus only a small number of proteins are being investigated by a small number of pharmaceutical companies at the moment (Kind and Schnieke, 2007).

ANTIBODIES
Proteins, produced by an organism in response to a pathogen or foreign substance, that neutralize that substance/ pathogen and help the immune system to eliminate the infection.

A particularly promising approach is the development of transgenic animals that express human polyclonal antibodies. **Antibodies** are the fastest growing set of new biopharmaceuticals, for therapeutic use in cancer, autoimmune diseases, infections, transplantations, biodefence and immune deficiencies. Currently all approved therapeutic antibodies are produced by cell culture techniques.

The possibilities for the production of polyclonal human antibodies in transgenic cattle are currently being investigated; such antibodies would mimic the natural human immune response to a pathogen. Cattle would be especially suited for this purpose, since the total amount of antibodies in an adult animal is approximately 1 kg. One approach towards this end is the use of artificial chromosomes to

transfer the human antibody genes to the target animal (Kuroiwa *et al.*, 2002). Concomitantly, the endogenous antibody genes of the animal are knocked out to prevent their expression and thus allow purification of human antibodies without contaminating bovine antibodies. To obtain human polyclonal antibody sera from the animal, the animal would need to be immunized with a vaccine containing the pathogen of interest, e.g. a bacterium or a virus. Subsequently, the animal would build up an immune response and express the human antibodies directed against that pathogen. These antibodies could subsequently be extracted and purified from the animal's blood plasma and used to treat humans suffering from an infection with that particular pathogen. This perspective for a quick availability of large amounts of human antibody sera targeted against a certain pathogen or disease agent has raised speculations about a transformation of medicine similar to the introduction of antibiotics in the 1940s and 50s (Kind and Schnieke, 2007). Similar approaches, based on the same methodology, are being pursued for the use of plants as bioreactors for the production of medically valuable proteins and small-molecule drugs (Twyman *et al.*, 2005).

5.4.3 Transgenic animals for improved disease resistance

Resistance or susceptibility to diseases and the immune response typically depend on a variety of genes, but identification of some key genes has brought up the possibility of gene transfer to target important and specific aspects of the immune system (Niemann *et al.*, 2005). Diseases that are under investigation, by either introducing resistance genes or removing susceptibility genes, include bovine spongiform encephalopathy (BSE), brucellosis, other viral or bacterial infections, parasitic organisms, and intrinsic genetic disorders.

One often-cited example is resistance against mastitis: mastitis is a bacterial infection of the bovine mammary gland, leading to decreased productivity and milk contamination. Transgenic cattle have been produced that secrete the small

protein lyostaphin in their milk, which is a potent inhibitor of *Staphylococcus aureus (S. aureus),* the bacterium responsible for the majority of mastitis cases. According to first trials, the transgenic cows are resistant to *S. aureus* – mediated mastitis (Donovan *et al.*, 2005).

Further approaches of animal transgenics target animal reproductive performance and prolificacy, development of organs for transplantations (xenotransplantation) that do not evoke a rejection response, or improvement of animal fibre and wool.

5.5 BIOTECHNOLOGY IN ANIMAL HEALTH

Apart from the aforementioned possibilities to generate transgenic animals with enhanced resistances to diseases, biotechnology offers a variety of other techniques that contribute to improved animal health. These include the production of vaccines to immunize animals against diseases, and the development of improved disease diagnostic tools.

5.5.1 Vaccines

VACCINES
A vaccine is a substance, derived from a pathogen, that is administered to an organism and stimulates the organism's immune system to prevent infection from that pathogen.

Vaccines are substances, derived from a pathogen, that are used to stimulate an animal's immune system to produce the antibodies needed to prevent infection from that particular pathogen. Vaccination is therefore the main approach to protect animals from infectious diseases. The majority of vaccines are based on material directly derived from inactivated bacteria or viruses, which potentially revert to their virulent (disease-causing) form. Modern biotechnology offers possibilities to engineer specific vaccines that are free from pathogen-derived material and are more effective and safe in stimulating the immune response (Rogan and Babiuk, 2005).

One approach is based on recombinant protein technology: once a protein from a pathogen that serves as antigen (i.e. a molecule that stimulates an immune response) has been identified, this protein can be safely expressed in cell culture,

e.g. in *E. coli* or mammalian cells, using recombinant DNA technology. Subsequently, this protein can be harvested, purified and used as a vaccine (also known as subunit vaccines). In addition, it has also become possible to create fusions of several pathogen proteins, so that one final protein stimulates a variety of immune responses (Meeusen *et al.*, 2007).

A second approach consists of using DNA-based vaccines. This methodology is based on the delivery of plasmid DNA to the cells of a host animal that encodes pathogenic proteins. Once expressed within the cell, the proteins stimulate the animal's immune response in the same way as if the proteins were delivered from outside; thus the animal serves as its own bioreactor for vaccine production (Rogan and Babiuk, 2005). The efficiency of this method is largely dependent on effective plasmid delivery to the animal cells; methods for delivery include chemical transformation, electroporation, injection and the gene gun.

A third approach is the delivery of pathogen-derived antigens by live recombinant vectors. Bacteria, viruses or even parasites can be engineered to express foreign proteins from the pathogen of interest that act as antigens. The engineered organism is then delivered to the animal, where it induces a limited infection and presents the foreign pathogenic protein, thus stimulating an immune response against that pathogen.

Recently, a very interesting combination of transgenic plant technology and animal vaccination has emerged: plants are engineered to express an antigenic protein from a pathogen at high levels in their tissues or storage organs. Subsequently these plants can be fed to animals and the vaccine is presented to and taken up by the mucosal surfaces in the intestine, thus providing a direct feed-vaccination (Floss *et al.*, 2007).

In addition to the vaccine itself, substances that stimulate vaccine uptake and activity (so-called adjuvants) and the route of vaccine delivery (injection, inhalation, feed, etc.) are factors that are strongly investigated and further developed by biotechnological methods.

5.5.2 Diagnosis of disease and genetic defects

Successful control of a disease requires accurate diagnosis. Modern biotechnology offers many applications to diagnose diseases caused by pathogens as well as diseases caused by intrinsic genetic disorders of an organism. The currently available and deployed techniques are outlined below.

The ability to generate highly specific antigens by recombinant DNA techniques has significantly raised the number of ELISAs that have the capacity to differentiate between immune responses generated by vaccination from those due to infection. This has made it possible to overcome one of the major drawbacks of antibody detection tests: the fact that, because antibodies can persist in animals for long periods, their presence may not indicate a current infection (Rege, 1996).

The advent of PCR has enhanced the sensitivity of DNA detection tests considerably. For example, PCR used in combination with DNA hybridization analysis has been shown to provide a sensitive diagnostic assay to detect bovine leukosis virus. This holds true for many other pathogenic organisms that are difficult to detect by serological methods (Schmitt and Henderson, 2005).

Other diagnostic techniques include nucleic acid hybridization assays and restriction endonuclease mapping. A good example of the specificity of nucleic acid hybridization is its application in distinguishing infections caused by *peste des petits ruminants* (PPR) virus from *rinderpest*, diseases whose symptoms are clinically identical and which cannot be distinguished with available serological reagents. This technique also allows comparison of virus isolates from different geographical locations.

Molecular epidemiology is a fast growing discipline that enables characterization of pathogen isolates (virus, bacteria, parasites) by nucleotide sequencing, allowing the tracing of their origin. This is particularly important for epidemic diseases, where

the possibility of pinpointing the source of infection can significantly contribute to improved disease control. Furthermore, the development of genetic probes, which allow the detection of pathogen DNA/RNA (rather than host antibodies) in livestock, and the advances in accurate, pen-side diagnostic kits can considerably enhance animal health programmes (FAO, 2001).

DNA testing is also being used to diagnose hereditary weaknesses of livestock. One available test identifies the gene which is responsible for Porcine Stress Syndrome in pigs. Animals that carry this gene tend to produce pale, low-quality meat when subjected to the stress of transport or slaughter. The identification of pigs that carry this gene excludes them from breeding programmes, resulting in an overall decrease in the frequency of that gene within a population (Madan, 2005).

DNA TESTING
Testing an animal for the occurrence of specific gene versions, and thus preferably use or exclude that animal from breeding programmes.

Another example of DNA analysis is the diagnosis of a mutation of Holstein cattle that causes leucoyte adhesion deficiency. Cattle with this condition suffer diseases of the gum, tooth loss and stunted growth. The disease is fatal, and animals usually die before reaching one year of age. The available test identifies carriers of the defective gene, allowing the elimination of such animals from breeding herds. Ideally, all animals used for breeding should be tested to exclude any carriers of the gene (Madan, 2005).

5.6 DNA TECHNOLOGIES IN ANIMAL NUTRITION AND GROWTH

5.6.1 Nutritional physiology

Applications are being developed for improving the performance of animals through better nutrition. Specific enzymes can chemically modify feedstuffs and thus improve the nutrient availability and uptake by the animal. This lowers feed costs and reduces output of waste into the environment. Prebiotics (substances that stimulate microbial growth) and probiotics (live micro-organisms) as feed

additives or immune supplements can either stimulate growth of beneficial micro-organisms in the digestive system, or inhibit pathogenic gut micro-organisms and render the animal more resistant to them. Administration of the recombinantly produced growth hormone somatotropin (ST) results in accelerated growth and leaner carcasses in meat animals and increased milk production in dairy cows. Immunomodulation, i.e. administration of substances that stimulate or repress immune system function, can be used for enhancing the activity of endogenous anabolic hormones (FAO, 2001).

In poultry nutrition, possibilities for improvement include the use of feed enzymes, probiotics and antibiotic feed additives. The production of tailor-made plant products for use as feeds that are free from anti-nutritional factors through recombinant DNA technology is also a possibility.

Plant biotechnology may produce forages with improved nutritional value or incorporate vaccines or antibodies into feeds that may protect the animals against diseases (see 5.5.1).

5.6.2 Rumen biology

Rumen biology has the potential to improve the nutritive value of ruminant feedstuffs that are fibrous, low in nitrogen and of limited value for other animal species. Biotechnology can alter the amount and availability of carbohydrate and protein in plants as well as the rate and extent of fermentation and metabolism of these nutrients in the rumen (FAO, 2001).

Methods for improving rumen digestion in ruminants include the use of probiotics, which is the supplementation of animal feed with beneficial live micro-organisms, to improve the intestinal microbial balance for better utilization of feed and for good health (Weimer, 1998). The added bacteria may improve digestion of feed

and absorption of nutrients, stimulate immunity to diseases, or inhibit growth of harmful micro-organisms. Transgenic rumen micro-organisms (see Chapter 6) could also play a role in the detoxification of plant poisons or inactivation of antinutritional factors. Successful introduction of a caprine rumen inoculum into the bovine rumen to detoxify 3-hydroxy 4(IH) pyridine (3,4 DHP), a breakdown product of the non-protein amino acid mimosine found in *Leucaena* forage is an example (Rege, 1996).

To conclude this chapter, it should be noted that many biotechnological applications are already available in the field of animal production and utilization. However, all techniques that have been successfully adopted so far are based on conventional biological methodologies, such as assisted reproduction and MAS. On the contrary, the approval and commercialization of techniques based on the creation of GM animals is only beginning to emerge. This is in sharp contrast with the field of transgenic plants, which have been in commercial use since the mid-1990s. Nevertheless, research in the field of GM animals is actively searching for solutions to the problems that are still linked to the production and application of GM animals. The approval of the first drug that is produced in a transgenic organism is a positive sign in this respect, and many other applications of GM animals, both in agriculture and medicine, are envisaged to follow in the near future.

CHAPTER 6

GENETIC ENGINEERING OF MICRO-ORGANISMS OF INTEREST TO AGRICULTURE

6.1 INTRODUCTION

MICRO-ORGANISM
All organisms that are not visible to the naked eye; including bacteria, archae, fungi, protists, green algae and small animals.

Micro-organism is a term employed to cover all organisms that are not visible to the naked eye; this includes bacteria, archae, fungi, protists, green algae and small animals, such as plankton. The development of genetically modified micro-organisms of interest to agriculture is of significant importance. These micro-organisms may be used as gene transfer systems or donors and recipients of desirable genes. Micro-organisms functioning as gene transfer systems and as donors of genes have already been discussed (see previous chapters). The focus of this chapter is therefore on microbial recipients of transgenes to obtain organisms with novel traits and properties.

Micro-organisms play important roles in different sectors of agriculture, food processing, pharmaceutical industries and environmental management. This development already started early in the history of humankind with the use of micro-organisms for the fermentation process. In the early 1970s, micro-organisms, notably *E. coli*, were used at the forefront of molecular biology research, resulting in the advent of recombinant DNA technology. The first recombinant protein, produced in a micro-organism and approved as a drug by the FDA in 1982, was human insulin. Since then hundreds of recombinant proteins have been engineered and expressed in micro-organisms

and approved for use as pharmaceuticals. Nowadays, many microbial processes and pathways are understood and deciphered at the genetic level and can thus be subjected to specific and targeted genetic manipulation (Bull *et al.*, 2000). Traditionally this approach largely depended on the identification and selection of random mutants with desirable characteristics; recombinant DNA technology presents a significant advance in this respect, since specific metabolic pathways can be manipulated with high precision and completely new functions can be introduced into an organism. The following sections give some examples of micro-organisms of economic importance that have been genetically modified through recombinant DNA technology.

The techniques underlying the production of genetically modified micro-organisms are basically the same that we encountered throughout the previous chapters. However, genetic engineering of micro-organisms is in many respects much easier compared to plants and animals (Demain and Adrio, 2008). Since many micro-organisms are single-cell organisms, they can be easily grown in cell culture in the laboratory in large quantities. Furthermore, DNA can be easily introduced using a variety of techniques, and it is obviously not required to reconstitute a complete transgenic organism from a transformed cell, as is the case with plants and animals. Many micro-organisms can grow under a variety of different conditions and with different nutrient sources and survive periods of unfavourable growth conditions. Furthermore, the genetic makeup and function of many micro-organisms are known in detail and are in general less complicated compared with multicellular organisms, facilitating targeted genetic manipulations.

6.2 GENETICALLY MODIFIED MICRO-ORGANISMS AS BIOPESTICIDES AND BIOFERTILIZERS

BIOPESTICIDES
Substances derived from organic material that exhibit pesticidal activity.

Biopesticides are defined as all substances derived from natural materials, including plants, animals and micro-organisms, that exhibit pesticidal activity. Such biological control agents are increasingly targeted for genetic enhancement due to a rising

recognition of their potential benefits to modern agriculture (Rizvi *et al*., 2009). Biological control represents an alternative to chemical pesticides which have been subjected to much criticism due to their adverse impacts on the environment and human health. Therefore, there is a strong requirement to develop safer and environmentally amenable pest control using existing organisms in their natural habitats. Several such organisms, referred to as biological control agents, are available that offer protection against a wide range of plant pests and pathogenic microbial agents without damaging the ecosystem.

If biological control agents are to be effective in plant disease management, they must be efficacious, reliable and economical (Fravel, 2005). To meet these conditions superior strains are often required that are not found in nature. In this case the existing attributes of the biocontrol agents can be genetically manipulated to enhance their biocontrol activity and expand their impact spectrum.

The foreign genes used for transforming biological control agents can be integrated into the host genome or a plasmid. To express a heterologous gene in fungi or bacteria, the regulatory region of this gene must be modulated in its promoter and terminator regions in order to optimize the expression of the inserted gene in the new host. The addition of specific genes that are known to confer biocontrol activity may enhance or improve biocontrol capacity of organisms that do not naturally possess these genes.

Free-living bacteria associated with plants have been targeted to enhance their capacity either as soil inoculants or as biocontrol agents of plant pathogens. Studies on micro-organisms capable of enhancing plant growth have concentrated on the rhizosphere (root zone) whereas those on biocontrol target both the rhizosphere and phylloplane (leaf zone). Several important rhizobacteria including *Sinorhizobium meliloti* and *Pseudomonas putidrii,* both of which are excellent root colonizers, lack the ability to synthesize chitinases. Chitinases are enzymes that destroy chitin, a

major component of fungi cells (Dahiya *et al.*, 2006). Introducing genes encoding chitinases into their genome have enabled them to provide protection against plant pathogenic fungi. These two bacteria are good targets because of the unique beneficial characteristics they confer. *Sinorhizobium* is a symbiotic bacterium which stimulates formation of root nodules in legumes involved in fixing atmospheric nitrogen. Many *Pseudomonas* species in the rhizosphere environment produce siderophores which chelate iron ions, thereby increasing iron uptake by plants. The genetically modified commercial strain (RMBPC-2) of *Sinorhizobium meliloti* has added genes that regulate the nitrogenase enzyme involved in nitrogen fixation (Scupham *et al.*, 1996).

The *Trichoderma* species are widely present in soils and are antagonistic to other fungi. *T. harzianum,* in particular, is a strong rhizosphere colonizer which is also able to parasitize plant pathogenic fungi. It establishes tight physical contact with hyphae of target fungi with the aid of binding lectins. Several extracellular enzymes, including chitinases, glucanases, lipases and proteases, are produced by the *Trichoderma* species, which has been improved further with the transfer of chitinase genes, notably from *Serratia marcescens* (Benitez *et al.*, 2004).

The *Agrobacterium radiobacter* strain k84 protects plants against crown galls caused by *A. tumefaciens* strains carrying Ti-plasmids of the nopaline type. Protection conferred by *A. radiobacter* strain k84 is due to agrocin 84, an A nucleotide derivative. When taken up by *A. tumefaciens,* it inhibits DNA synthesis, resulting in cell death (Vicedo *et al.*, 1993). *A. radiobacter* has an additional negative effect on soil pathogens by being a very effective rhizosphere colonizer. Although *A. radiobacter* strain k84 has been widely used commercially for a long time, there was concern about its long-term effectiveness as a biocontrol agent. This is because the gene encoding agrocin is carried on a transmissible plasmid, which can be transferred by conjugation to *A. tumefaciens.* In the event of agrocin-encoding plasmid transfer, recipient *A. tumefaciens* strains would no longer be subjected to biocontrol by *A. radiobacter* strain k84. This concern was addressed by modification of the agrocin-encoding plasmid to prevent its transfer

to *A. tumefaciens*. The ensuing genetically engineered strain, known as *A. radiobacter* strain K1026, is a transgenic organism approved for use as a pesticide (EPA).

BACILLUS THURINGIENSIS
One of the best-known and adapted biopesticides, active against a wide range of insect pests.

Bacillus thuringiensis (Bt) has been used as a biopesticide for many years. The insecticidal activity of *B. thuringiensis* is based on the production of crystalline protein inclusions during sporulation. The crystal proteins are encoded by different cry genes and are also known as delta-endotoxins. The protein crystals are highly toxic to a variety of important agricultural insect pests; when the proteins are taken up by susceptible insect larvae they induce lysis of gut cells, resulting in death of the larvae by starvation and sepsis (Roh *et al.*, 2007). The toxin can be applied to plants as a spray consisting of a mixture of spores and protein crystals. However, the toxin has the disadvantage of fast degradation in sunlight. To overcome this limitation, different cry genes encoding the Bt toxin have been cloned and introduced into another bacterium, *Pseudomonas flourescens*. The transgenic *P. flourescens* strains are killed and used as a more stable and persistent biopesticide compared to the *B. thuringiensis* sprays (Herrera *et al.*, 1994). Furthermore, cry genes are widely used to create transgenic plants that directly express the toxin and are thus protected from susceptible insect pests (see Annex 1.2.2).

Baculoviruses (although, per definition, viruses are not micro-organisms) are also being manipulated to be effective biopesticides against insect pests such as corn borer, potato beetle and aphids (Szewczyk *et al.*, 2006).

6.3 MICRO-ORGANISMS FOR ENHANCING THE USE OF ANIMAL FEEDS

Animal digestive tracts harbour beneficial microflora that aid in the digestibility of various feeds. However, the function of these micro-organisms is easily affected by the unfavourable conditions within the gut, such as acidity and antibiotics used to treat pathogenic micro-organisms. Examples of gut micro-organisms that have been genetically modified include *Prevotella ruminicola* with a tetracycline

resistance gene, cellulolytic rumen bacteria with acid tolerance, hind gut bacteria with cellulose activity, rumen bacteria transformed with genes to improve protein yield and yeast (*Saccharomyces cerevisiae)* containing a transgene from the closely related *Saccharomyces diastaticus,* allowing it to increase the digestibility of low-quality roughage in conventional feeds (Weimer, 1998). The major limitation to the use of these engineered organisms has been their establishment in the appropriate regions of the gut. Some organisms are being used as beneficial supplements in animal feeds. These are called **probiotics** and their use aims at improving digestion of feed and absorption of nutrients, stimulate immunity to diseases and inhibit growth of harmful micro-organisms (Gomez-Gil *et al.*, 1998). For the improvement of silage, strains of the bacterium *Lactobillus planetarium* are being developed with the aim of increasing the lactate content and reduce the pH and ammonia content.

PROBIOTICS
Live micro-organisms as feed supplies to improve digestion, nutrient uptake and immune function.

Micro-organisms are being extensively used as bioreactors for the production of hormones and other substances that enhance animal size, productivity and growth rates. The recombinantly produced hormone bST (bovine somatotropin) was among the first recombinant hormones commercially available. It can increase milk yield by as much as 10 to 15 percent when administered to lactating cows (Etherton and Bauman, 1998). Current development efforts are looking at a wide spectrum of genes that affect growth and productivity within the animal and which could be expressed in recombinant micro-organisms to obtain the respective protein in large quantities.

6.4 GENETICALLY MODIFIED MICRO-ORGANISMS IN FOOD PROCESSING

Many micro-organisms are being manipulated with the objective of improving process control, yields and efficiency as well as the quality, safety and consistency of bioprocessed products. Modifications target food enzymes, amino acids, peptides (sweeteners and pharmaceuticals), flavours, organic acids, polysaccharides and vitamins. A classical example is the production of the recombinant cheese making enzyme, chymosin, in

bacteria. Its use was approved in 1990 in the United States, and nowadays 80 percent of US cheese is produced using this product (Law and Mulholland, 1991).

6.5 GENETICALLY MODIFIED MICRO-ORGANISMS IN BIOREMEDIATION

Micro-organisms are widely used in cleaning up pollution such as oil spills or agricultural and industrial wastes by degrading them into less toxic compounds (Chatterjee *et al.*, 2008). Some bacteria are being used as "bioluminescensors" that give luminescence in response to chemical pollutants. An example is the mercury resistance gene *mer* that is expressed in some bacteria and can result in bioluminescence upon encountering the presence of even very low levels of mercury in the environment.

A modified bacterium, *Rhodopseudomonas capsulate,* has the ability to grow rapidly in simple synthetic media. It is being used in advanced swine waste treatment plants in both Japan and Republic of Korea. The concentration of short chain fatty acids, one of the main sources of the bad odour of swine wastes, decreased dramatically after treatment. The residue after treatment can be used as a safe organic fertilizer. Several other applications of micro-organisms or plants for the purpose of **bioremediation** are being investigated.

BIOREMEDIATION
The use of living organisms to detoxify or remove pollutants from the environment.

To conclude this chapter, micro-organisms have always been at the forefront of research and development in the field of recombinant DNA methodology and biotechnology. As mentioned in the introduction to this chapter, this can be largely attributed to the comparative ease of culturing, analysing and manipulating many micro-organisms. Nevertheless, many micro-organisms and their potential benefits remain unexplored and new species are being discovered regularly; therefore, research and development of biotechnological applications for micro-organisms in the field of agriculture and nutrition holds great promise for the future (Bull *et al.*, 2000).

CHAPTER 7

GMO DETECTION, IDENTIFICATION AND QUANTIFICATION METHODS

7.1 INTRODUCTION

The precise and accurate detection of GMOs with high sensitivity in a given biological sample is of significant importance. This need for exact **GMO detection** methods will become increasingly clear in the following modules, when concepts for GMO surveillance, monitoring, biosafety measures and the implementation of relevant regulations are introduced.

GMO DETECTION
The process of detecting GMO material in a given sample.

Different stakeholders involved in the development, use and regulation of GMOs do at some point need to monitor and verify the presence and the amount of GMO material in agricultural products. Furthermore, comprehensive GMO monitoring also includes the analysis of biological samples, such as material derived from plant species that are related to an introduced GMO, to check for horizontal transfer of the transgene. This need has generated a demand for analytical methods capable of detecting, identifying and quantifying either the unique DNA sequences introduced or the protein(s) expressed in transgenic plants and animals. Thus, comprehensive GMO analysis techniques consist of three steps: detection, identification and quantification of GMO material (Anklam *et al.*, 2002).

- **Detection (screening for GMOs).** The objective of this first step is to determine if a product contains GMO material or not. For this purpose, a screening method can be used. The result is a qualitative positive/negative statement. Analytical methods for detection must be sensitive and reliable enough to obtain accurate and precise results and reliably identify small amounts of GMO material within a sample.
- **Identification.** The purpose of the identification step is to reveal how many different GMOs are present in a sample, to precisely identify each single one and determine if they are authorized or not. Specific information (i.e. details on the molecular make-up of the GMOs) has to be available for the identification of GMOs.
- **Quantification.** If a food product has been shown to contain one or more authorized GMOs, it becomes necessary to assess compliance of the set threshold level regulations for the product in question. This is achieved by determining the exact amount of each GMO that has been found in the sample.

GMO TESTING FRAMEWORK
Comprehensive GMO analysis consists of GMO detection, GMO identification and GMO quantifiifcation.

This **testing framework** is depicted in Figure 7.1, with labelling regulation thresholds of the European Union (adapted from Anklam *et al.*, 2002).

In general, the range of sample types that need to be tested for GMO content is extensive and covers raw commodities as well as highly processed food. Furthermore, the number and variety of worldwide commercially grown GMOs is constantly increasing. Therefore, it is necessary to carefully approach each sample on a case-by-case basis and thus determine the most appropriate testing method (Jasbeer *et al.*, 2008)

Every method developed for the detection of GMOs that is considered for routine use by official testing authorities and laboratories has to undergo several testing procedures to verify the analytical performance of the method (Michelini *et al.*, 2008). The performance requirements of each method include applicability (if it is suited for the detection purpose), practicability (costs, material and machine

requirements), specifity, dynamic range (range of different concentrations that can be detected), accuracy, limits of detection and quantification, and robustness (reproducibility of results) (Lipp *et al.*, 2005).

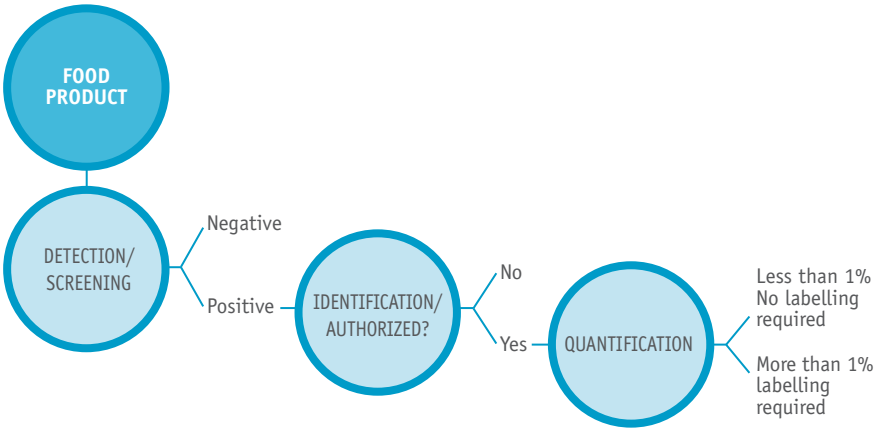
7.2 SAMPLING PROCEDURES

SAMPLING PROCEDURES
Prior to the actual analysis, the sampling procedure has major importance for the outcome and statistical value of a GMO analysis procedure.

Irrespective of the analytical method selected for GMO detection, correct **sampling procedures** are critically important for reliable and reproducible GMO analysis. An insufficient sampling plan can have strong effects on the reliability of the detected GMO level. In fact, the variance associated with the sampling procedure likely represents the major contribution to the overall variance of the detection procedure (Michelini *et al.*, 2008). Furthermore, GMO material usually shows a heterogeneous distribution within the bulk of a product, additionally contributing to sampling-dependent variance. Raw materials, in particular, may show a significant

Figure 7.1 | **GMO detection framework**

A comprehensive testing scheme consists of GMO detection/screening, GMO identification and GMO quantification.



heterogeneity, whereas processed materials and food usually display a more uniform distribution. The influence of the sampling strategy is more relevant when the overall GMO concentration is low.

Samples must therefore be taken in a manner that ensures that they are statistically representative of the larger lot volume or quantum of material. The sample size has to be adjusted to the required sensitivity and allow reliable GMO detection; the smaller the sample, the weaker the statistic significance of the testing procedure. So far, no generally accepted sampling guidelines have been established, and different control authorities employ different sampling schemes (Anklam *et al.*, 2002). The major parameters that influence the sampling plan are lot size, lot heterogeneity, the defined tolerance level and the applied testing methods. Furthermore, parameters that are specific for each event, i.e. the size of the host genome, the copy number of the transgene event involved, and the amount of material that can be analysed in a single test, need to be taken into consideration (Lipp *et al.*, 2005). Efforts are underway to define and internationally harmonize sampling plans, based on sound statistical requirements and analyses (Miraglia *et al.*, 2004).

An example of a sampling plan, based on kernels, is calculated by Grothaus *et al.*, 2006:

- To detect a lot concentration of 0.01 percent GMO material with 99 percent probability, 46 050 particles are required.
- To detect a lot concentration of 0.1 percent GMO material with the same confidence of 99 percent, 4 603 particles are required.
- If the confidence level for the detection of 0.1 percent GMO material is decreased to 95 percent, 2 995 articles are required.

Other calculations based on kernels state that at least 3 500 particles are required to detect a 1 percent contamination with a confidence level of 95 percent (Ovesna *et al.*, 2008). The International Organization for Standardization (ISO) has issued

a brochure on sampling procedures for GMO testing (ISO, 2006); a handbook from the International Seed Testing Agency (ISTA) on this topic is also available (ISTA, 2004). However, it should be noted that in any case the sampling strategy is highly dependent on the material analysed (raw, processed ingredients and processed food) and the required sensitivity, and it should be revised on a case-by-case basis. The establishment of a sampling plan that takes into account all relevant parameters and factors is a complex statistical procedure (refer to Remund *et al.*, 2001 for further information). The reduction of sampling errors and thus more reliable test results are important for all involved parties: for consumers, the probability of consuming food that has been accepted although containing GMO above set **threshold limits** is reduced, and for producers the probability of lot rejection although the GMO content is below the set threshold limit is reduced as well. Therefore, the adoption and implementation of standardized sampling procedures should be of interest to all parties involved in GMO production, trading and consummation (Miraglia *et al.*, 2004).

THRESHOLD LIMITS
Defined limits of GMO content that are allowed in a given product, and that needs to be labeled accordingly.

The actual sampling procedure consists of various steps: (1) sampling the lot of seed, grain or other material to obtain the bulk sample; (2) sampling the bulk sample to obtain the laboratory sample; (3) subsampling the laboratory sample to obtain the test sample; (4) homogenization (grinding etc.) of the test sample and sampling of the resulting meal to obtain the analytical sample; (5) extracting the analyte of interest (DNA, protein) from the analytical sample and using subsamples of it as final test portions (Lipp *et al.*, 2005). The final test portion, for example in the case of PCR analysis of DNA, is typically around 100-200 ng of DNA which can be used in a single PCR.

7.3 SAMPLE PREPARATION PROCEDURES

The next step in GMO detection and quantification analyses, following the sampling procedure, is sample preparation for subsequent analytical procedures. Since all

officially approved detection techniques rely on either DNA or protein-based assays, this section will focus on sample preparation and extraction techniques for these two compounds.

SAMPLE PREPARATION Extracting the analyte, usually DNA or protein, from a sample for subsequent analyses.

The ultimate aim of **sample preparation** is the isolation of DNA or protein with sufficient integrity, purity and quantity to allow reliable detection and quantification analyses. The choice of extraction procedure depends on the sample matrix, the target analyte and the type of analysis to be performed (GMO screening, identification or quantification). Different sample matrixes in combination with different extraction procedures have been shown to strongly influence the outcome of subsequent analyses (Cankar *et al.*, 2006), therefore the appropriate extraction method needs to be determined for each individual sample (Jasbeer *et al.*, 2008).

A further complication is the fact that samples often consist of highly processed food, i.e. the original plant or animal material has undergone several manufacturing steps. This might include simple mechanical procedures, such as milling, or complex chemical or enzyme-catalysed modifications. Since proteins and DNA are likely to be degraded during such processing steps, the detection of these compounds in highly processed food requires sensitive and reliable detection methods (Michelini *et al.*, 2004).

7.3.1 DNA extraction procedures

Compared with protein, DNA is a relatively stable molecule that can still be identified when it is partially degraded or denatured, contributing to its prime importance for GMO detection. It is possible to obtain DNA suitable for subsequent analyses from highly processed and refined food matrices; examples of failures to isolate DNA, to date, include refined soybean oil, soybean sauce and refined sugar (Jasbeer *et al.*, 2008). DNA can be isolated as intact, high molecular weight DNA from fresh material, or as fragmented DNA from processed, old material (Ovesna *et al.*, 2008).

Three parameters are characteristic for DNA extraction procedures:

- The DNA quantity: the overall amount of extracted DNA.
- The DNA quality: as mentioned, food processing has a negative effect on DNA quality. Heat exposure, enzymatic degradation or unfavourable chemical conditions contribute to DNA fragmentation or damage. Target sequences for subsequent analyses, therefore, often do not exceed 100-400 bp in length.
- DNA purity: DNA in food matrices might be severely contaminated, by substances such as polysaccharides, lipids or polyphenols. Obtaining DNA of high purity is important to avoid complications or misleading results during subsequent analyses.

The key steps in sample preparation include homogenization of the material, chemical or enzymatic pretreatment, extraction and purification (Jasbeer *et al.*, 2008). Concerning plant material, small aliquots of 100-350 mg are sufficient for DNA isolation, given that this laboratory sample is representative of the field sample and has been correctly homogenized (Anklam *et al.*, 2002).

Five DNA extraction methods are commonly used, depending on the food matrix to be analysed. These are the DNeasy Plant Mini Kit (Qiagen), Wizard extraction (Promega), GENESpin Kit (GeneScan), cetyl trimethylammonium bromide (CTAB) based extraction, or a combination of CTAB-extraction with DNA-binding silica columns (Michelini *et al.*, 2008). It is important to carefully determine the extraction method that is most suited for the food matrix in question in order to obtain reliable and reproducible extraction and analysis results.

7.3.2 Protein extraction procedures

In contrast to DNA, proteins are very heat-labile molecules. Furthermore, they are easily affected by chemical treatments or enzymatic degradation. The detection of a specific protein depends on the recognition of this protein by an antibody

directed against that protein. If the target protein is degraded or denatured (i.e. loses its specific 3-dimensional shape), this antibody-mediated detection can no longer be performed. Therefore, it is not possible to reliably and reproducibly detect and quantify proteins in complex food matrices, such as processed agricultural material and food products, that have been subjected to mechanical, thermal, enzymatic or chemical processing (Anklam *et al.*, 2002).

Due to these limitations, protein analysis is only applicable for materials in their raw state (Jasbeer *et al.*, 2008). However, the basic steps in sample preparation are the same as in DNA extraction: material homogenization, pretreatment, extraction and purification.

7.4 GMO DETECTION BY PHENOTYPIC CHARACTERIZATION

Phenotypic characterization is possible if the inroduced transgene(s) result in the absence or presence of a specific trait that can be screened by analysing the phenotype of the organism. Detection methods using this approach are referred to as bioassays. This approach can be used, for example, to test for the presence or absence of herbicide resistance transgenes. One such test is based on the germination of seeds in the presence of the herbicide of interest and subsequent analysis of germination capacity. Herbicide assays are considered to be accurate and inexpensive. Controls, including seeds with or without the trait targeted, should be included in all samples tested. Typically, a test sample consists of 400 seeds. The test accuracy is dependent on the overall germination efficiency of the seeds: the higher the germination efficiency, the higher the confidence level of the test. Obviously, only viable seed or grain can be tested (no processed products), and each test requires several days to complete. Furthermore, bioassays require separate tests for each trait in question and at present such tests will not detect non-herbicide tolerance traits. Therefore, the tests are only of limited value for inspection authorities.

7.5 MOLECULAR DETECTION AND QUANTIFICATION OF GMOs – DNA-BASED METHODS

As stated above, the methods of choice for detecting and quantifying GMO material on a molecular level are based on detecting either the inserted, foreign DNA fragments or the novel proteins that are expressed from this DNA. Methods for the detection of foreign DNA rely mainly on PCR (Box 7.1), that allows amplification and detection of specific DNA fragments from the entire genome. Another advantage of **DNA-based detection** is the finding that there is usually a linear relationship between quantity of GMO present in a sample and quantity of transgenic DNA, thus it can be used to accurately quantify the amount of GMO material present in a sample. Finally, the stability of DNA and the extractability of suitable DNA even from highly processed food matrices contribute to its prime importance for GMO analysis.

DNA-BASED GMO DETECTION
Detection of GMOs via DNA is the most commonly used and most reliable technique. The modified DNA is detected by PCR.

7.5.1 PCR-based GMO detection

As evident from the name, GMOs are the result of genetic modification. Therefore, the most suitable GMO detection methods are those that directly target the modification itself – the modified DNA.

Polymerase chain reaction (PCR), including variants of the technique such as competitive PCR and real-time-PCR, is the method of choice for DNA-based GMO detection, identification and quantification (Lipp *et al.*, 2005). Due to its very high sensitivity, PCR is well suited for the analysis of processed food matrices containing degraded DNA or material that has only low GMO content.

PCR-BASED GMO DETECTION
Detecting GMOs by amplifying sequences of the introduced transgene by PCR. Requires knowledge of the molecular makeup of a GMO.

PCR-based GMO detection is dependent on detailed knowledge of the molecular makeup of a GMO, i.e. the sequence of the transgene and, optimally, the transgene integration site in the host genome. For authorized and commercially released GMOs, such information is available in public databases such as AGBIOS

(Ovesna *et al.*, 2008). In general, a typical gene construct for the production of a GMO consists of at least three elements (refer to Chapter 1): a promoter to drive expression of the inserted gene(s), the inserted/altered gene(s), and a terminator as a stop signal behind these genes. Such sequences can be specifically detected in a PCR analysis.

If no detailed sequence information about a GMO is available, PCR-based methods rely on the detection of commonly used genetic elements. Such frequently used elements are, for example, the CaMV 35S promoter, the *A. tumefaciens* nopaline synthase terminator (nos3'), or the kanamycin resistance marker gene (nptII) (Michelini *et al.*, 2008). Focusing on such sequences for routine GMO screening purposes is promising, since many commercially available GMOs contain these elements, or varieties thereof, and can thus be detected in standard screening procedures.

GMO detection is frequently based on the detection of the P-35S and nos3' genetic elements; however, several approved GMOs do not contain the P-35S or nos3' sequences and additional target sequences are needed to detect their presence. Furthermore, to detect as many variants of a GMO marker as possible (there are at least eight variants of P-35S used in GM crops), a careful choice of primers (see Box 7.1) is required. In addition, it should be noted that the detection of a common GMO marker solely indicates the presence of material derived from a GMO within a sample, but does not provide any information about the species or the engineered trait (Jasbeer *et al.*, 2008).

Most PCR-based GMO detection methods include a positive control primer set for the amplification of a reference gene. This is often a so-called housekeeping gene, which is present in (and unique to) all varieties of the investigated species (Miraglia *et al.*, 2004). Examples include the lectin gene in soybean or the invertase gene in maize. If a strong signal cannot be obtained with the positive control primer set, then there may be problems with the integrity or purity of the extracted DNA.

BOX 7.1

THE POLYMERASE CHAIN REACTION (PCR)

The **Polymerase chain reaction (PCR),** developed in the early 1980s, allows the million-fold amplification of a specific target DNA sequence. This is achieved by framing the target sequence with two primers, synthetic oligonucleotides of 20 to 30 bp, that are complementary to either one end of the two strands of the target sequence. For primer design, exact knowledge of the target sequence is required. Amplification of the target sequence is achieved by elongation of these primers by an enzyme (a DNA polymerase) using the target sequence as template. Repeating this reaction several times results in an exponential accumulation of the target sequence, since the amount of target sequence is doubled during each reaction cycle.

In principle, the PCR is a multiple-step process with consecutive cycles of three different temperatures, where the number of amplified target sequence grows exponentially according to the number of cycles. In each cycle the three temperatures correspond to three different steps in the reaction.

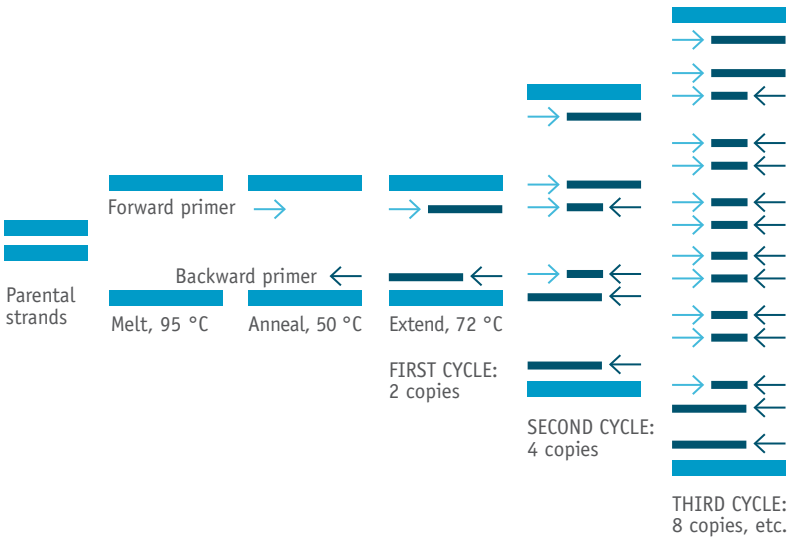
In the first step, the template DNA, i.e. the DNA serving as master-copy for the DNA fragment to be synthesized by DNA polymerase, is transformed from a double helix into single strands by heat denaturation at ~94 °C.

In the second step, the reaction mixture is cooled down to a temperature of 50-65 °C (depending on the primers used) to allow the annealing of the primers to both ends of the target sequence. Primer hybridization is favoured over DNA-DNA hybridization because of a high excess of primer molecules compared with template DNA in the reaction mixture. However, the annealing process is uncontrolled and can give rise to a large number of mismatched DNA duplexes.

POLYMERASE CHAIN REACTION
The process of rapidly amplifying a defined piece of DNA by repeated cycles of an enzymatic reaction.

Figure 7.2 | **The polymerase chain reaction**

See text for details.



In the third step, the annealed primers are extended using the target DNA strands as templates. This is usually performed by the enzyme DNA polymerase from the archaebacterium *Thermus aquaticus* (Taq) at its optimum temperature of 72 °C. With the elongation of the primers, a copy of the target sequence is generated.

The cycle is then repeated 20 to 50 times, depending on the initial amount of DNA present and the length of the amplicon (i.e. the amplified DNA fragment). The reaction results in an exponential amplification of the initially present target DNA, that can be subjected to subsequent analyses such as Southern blot, restriction digests or sequencing to verify its identity. In principle, PCR can be performed with as little as one template DNA initially present, but usually samples are adjusted to contain 25 to 100 template molecules. Due to this high sensitivity, PCR is very susceptible to contamination with undesirable DNA, that might produce false results.

Negative controls, for example samples with all necessary PCR ingredients but without template DNA, should also be included routinely to test for contamination with undesired DNA.

The **outcome of a PCR** can be evaluated by a variety of methods. Most frequently, amplified DNA fragments are subjected to agarose gel electrophoresis, a method to separate and visualize DNA fragments according to size. Since the expected size of a given target sequence is known, the presence of a fragment of that size indicates the presence of that target sequence in the original sample. If no fragment of the expected size is obtained, the sample did not contain the target sequence (given that the PCR worked well). To further verify the identity of an amplified fragment, it can be subjected to hybridization experiments with a complementary sequence, to analytical restriction enzyme digest, or to sequencing (Michelini *et al.*, 2008).

PCR EVALUATION
The outcome of a PCR can be most easily assayed by determining the size of the product. Other techniques are restriction enzyme digest, hybridization assays, or sequencing.

7.5.2 PCR-based GMO identification

Following a positive result from a GMO screening procedure, the next step is the unequivocal **identification of the GMO**(s) contained in a sample and the genetic modification event(s) involved. This can be achieved by PCR as well; however, compared with GMO detection, GMO identification is even more dependent on detailed information about the exact genetic modification of a GMO. In fact, this is a major limitation of PCR-based GMO detection and identification: if no such information is available, the GMO will not be detected or identified. Several approaches for GMO identification by PCR exist, and they are summarized below:

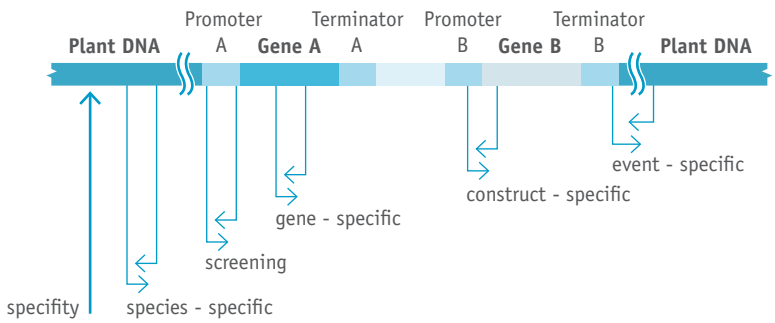
GMO IDENTIFICATION
Following GMO detection, the exact origin and GM species needs to be identified.

» *Gene-specific PCR:* In a gene-specific PCR, primers are used that lead to the amplification of a fragment from one gene of the transgenic element. This is rather unspecific, since many GMOs are engineered to contain the same, favourable genes. Thus, this method will fail to distinguish between these GMOs. This approach is therefore only useful if the target gene is present in only one GMO within a sample.

- *Construct-specific PCR:* This approach is more specific than gene-specific PCR. It is based on primers that target the junctions between different elements of the transgene insert, e.g. between the promoter and the gene or between different genes of the insert. Many GMOs contain identical genes, but the exact layout of their transgenes may differ, for example by a different arrangement of the genes or by the use of different promoters and terminators. By using construct-specific PCR, these different constructs, and thus GMOs, can be distinguished and identified.
- *Event-specific PCR:* Event-specific PCR is the most specific GMO identification strategy. Event, in this case, refers to the insertion of a transgene cassette into the host genome. The integration site is usually specific for each GMO. PCR primers, in this case, target the junction between the transgenic insert and the adjacent host genomic DNA. In most cases, this allows GMO identification with high certainty.

The different PCR layouts are also depicted in Figure 7.3. PCR evaluation is performed in the same way as described in 7.4.1, i.e. by visualization of amplified DNA fragments and subsequent sequence verification.

Figure 7.3 | **Different PCR strategies with increasing specificity**



Adapted from: Ovesna *et al.*, 2008.

Due to the dependency of PCR on detailed genetic information about GMOs there is a strong need for a continuous survey of all data available on GMOs – especially the introduced genetic elements and their integration sites. This applies not only for GM products approved for market release but also for any other GMO released for field trials worldwide. Only complete and accessible GMO information can guarantee comprehensive monitoring, detection and identification of GMOs.

7.5.3 PCR-based GMO quantification

The third step in GMO analysis, following detection and identification, is **GMO quantification**. Quantifying the GMO content in a sample is important to assess compliance with specific threshold levels for GMOs established by biosafety regulations. The typical approach to quantification utilizes one or more of the broad-spectrum primer sets that target common transgenic elements in GMOs. However, since different GMOs possibly contain these common elements in different numbers, accurate determination of GMO content cannot rely on the use of these common sequence elements alone. Quantification based on event-specific primers is therefore the most accurate means of obtaining quantitative results on GM content.

GMO QUANTIFICATION
Following GMO detection and identification, the exact amount of GMO material within a sample needs to be determined to check compliance with threshold and labeling regulations.

In general, two quantification approaches can be distinguished: absolute quantification and relative quantification. Absolute quantification, as the name suggests, yields absolute values of an analyte within a sample, e.g. how many milligrams of DNA could be extracted from a sample? This quantification is dependent on the sample size. The second approach is relative quantification: this is a measure of the amount of a substance compared to another substance, e.g. how many copies of transgene DNA per total DNA, or how many copy numbers of a gene per genome? Importantly, the final value obtained is a percentage, and the measurement is independent of the analysed sample size. Relative quantification is required for all GMO-related questions, such as compliance with labelling regulations (Jasbeer *et al.*, 2008).

7.5.3.1 Use of conventional PCR quantification

COMPETITIVE PCR
A varietation of PCR to quantifiy the amount of template DNA in a sample. Based on the co-amplification of a so-called competitor sequence.

One possibility for DNA quantification based on conventional PCR is double competitive PCR (DC-PCR). In **competitive PCR**, one primer pair is used to amplify both the target GMO template DNA and a synthetic template DNA fragment that is added to the same reaction mixture. The second fragment, which has a different size from the GMO target DNA (≤ 40 bp), is called the competitor. By conducting a series of experiments with varying amounts of the added synthetic DNA, it is possible to determine the amount of target GMO DNA in the sample. The competitor DNA serves as internal standard, and is added in different concentrations to the reaction mixture (an experimental setup known as titration). Following PCR amplification, the amplified fragments are visualized by agarose gel electrophoresis. The ratio of the two amplification products then represents the ratio of the initial two template sequences in the PCR mix. In other words, when the two products show equal amplification intensities, the amounts of initial template DNAs were the same. Since the amounts of added competitor DNA are known, this allows quantification of the target DNA in the sample.

Competitive and double-competitive PCR methods are semi-quantitative as a standard is required for comparison. In these cases the standard is the known amount of synthetic DNA. Consequently, the results will only indicate a value below, equal to or above a defined concentration of the standard.

7.5.3.2 Real-time PCR for GMO quantification

REAL-TIME PCR
A PCR format that allows quantification of the amounts of a specific DNA sequence within a sample.

Another strategy that improves accuracy, specificity and throughput of quantitative PCR is **real-time PCR**. This technique was originally developed in 1992 and is rapidly gaining popularity due to the introduction of several complete real-time PCR instruments and easy-to-use PCR assays. A unique feature of this PCR technique is that the amplification of the target DNA sequence can be followed during the

entire reaction by indirect monitoring of product formation. To this end, the conventional PCR reaction has been modified in order to generate a constantly measurable signal, whose intensity is directly related to the amount of amplified product. This signal is usually fluorescence, which is produced by an interaction between newly amplified DNA with certain added fluorophores. The increases in fluorescence during the reaction, that correspond to increasing concentrations of target DNA, are automatically measured, displayed on a computer screen, and can be analysed using suitable software.

DNA quantification by real-time PCR is based on the following principle: the PCR reaction mixture is submitted to several cycles of the reaction, until a fluorescent signal is encountered that is statistically significant above the noise level. The number of PCR cycles necessary to reach this threshold is recorded and referred to as Ct (cycle threshold) value. It is important to measure the Ct value in the exponential phase of the amplification procedure. During this stage, the Ct value is inversely proportional to the initial amount of template DNA molecules. In other words, a sample with many template molecules will reach a certain fluorescence threshold level faster than a sample with fewer molecules. For example, if a sample contains twice as many template molecules as a second sample, it will reach the threshold one cycle before the second sample since the amount of DNA is doubled during each reaction cycle. Thus, a low Ct value corresponds to a high initial concentration of target DNA.

Quantification of GMO DNA in a sample by RT-PCR is based on a combination of two absolute quantification values; one for the GMO target transgenic DNA and one for a species-specific reference gene. The GMO content in a sample can be calculated as a percentage using these two absolute values (Michelini *et al.*, 2008). Careful choice of suitable reference material is therefore of crucial importance for determining exact ratios of GMO to non-GMO material. Furthermore, it is important to know the copy number of the inserted transgenic sequences.

DETECTION LIMIT
Using real-time PCR, as little as 0.01 percent of GMO material can be reliably identified and quantified.

The **detection limit** of real-time PCR is very high; for corn, a detection of 0.01 percent GM corn versus non-GM corn has been demonstrated (Anklam *et al.*, 2002).

Several types of fluorescent probes for quantification of DNA using real-time PCR are currently available. One can discriminate between two classes of fluorophores: general DNA-binding dyes and fluorescent reporter probes. The first ones, a prominent example being SYBR Green, bind to double-stranded DNA in an unspecific manner and the resulting dye-DNA complex shows fluorescence. Since the overall amount of dsDNA in a PCR reaction increases, so does the intensity of fluorescence. The second type of probe consists of an oligonucleotide that is complementary to the target sequence, and a fluorophore and a quencher dye attached to it (e.g. the Taqman system). In the intact probe, the fluorophores' fluorescence is inhibited by the proximity of the quencher dye. During the annealing step of the PCR cycle, the oligonucleotide anneals to the target sequence between the two primers. Upon passage of the DNA polymerase during the elongation step, the oligonucleotide is cleaved and the fluorophore is liberated from the quencher dye. Thus, with increasing PCR cycles, the intensity of fluorescence increases as well. The latter, reporter-probe based method has the advantage that only the amplification of the desired target sequence is measured, while non-specific DNA binding dyes also react with non-specific PCR amplification products or other DNA hybrids (Miraglia *et al.*, 2004).

7.5.4 Confirmatory assays

CONFIRMATORY ASSAYS
All GMO detection, identification and quantififcation steps need to be verified by confirmatory assays to ensure the correctness of the obtained results.

Following PCR analysis, the identity of the amplicon needs to be confirmed and verified to ensure that the amplified sequence indeed represents the target sequence and is not an unspecific PCR artifact. Several **confirmatory assays** are available and commonly applied. Agarose gel electrophoresis, the simplest technique, can be applied to check if the amplicon is the expected size. However, it cannot be excluded that a PCR artifact, by coincidence, has the same size as the target sequence. To

further verify amplicon identity, it can therefore be subjected to restriction enzyme digest, since every DNA sequence has specific restriction profile. A further assay is Southern blotting, where the target amplicon is subjected to gel electrophoresis, transferred from the gel to a membrane, and hybridized with a complementary, labelled DNA probe; only the correct target sequence will yield a signal from binding of the complementary probe. A further possibility is nested PCR, where two primer pairs and two rounds of amplification are used: the second primer pair anneals within the target region of the first amplification, thus only the correct first amplification product will yield a second amplification product. The ultimate confirmatory assay is sequencing of the amplicon; however, this is rather expensive and requires special equipment that is not available in standard laboratories.

As stated above, PCR is able to amplify and thus identify very small amounts of initial target DNA. This implies that PCR is very sensitive to contamination with undesired DNA, possibly yielding false results in subsequent analyses. Therefore, high caution must be taken during all steps of PCR sample preparation and reaction setup to avoid cross-contamination. This already begins at sampling and sample preparation: it might already be sufficient to use the same grinding device for homogenization of two samples to produce contamination, even if no visible traces were left. Therefore it is of major importance to thoroughly clean and monitor all devices that come in contact with samples and that could potentially contribute to cross-contamination.

7.6 MOLECULAR DETECTION AND QUANTIFICATION OF GMOs – PROTEIN-BASED METHODS

A GMO is typically characterized by the introduction of novel genes, which direct the expression of novel proteins. Therefore, the second approach to detect GMOs is not based on detection of the modified DNA, but on the novel and newly expressed proteins. However, whereas modified DNA can be detected in all parts

of a transgenic organism at all times, this may not be the case for proteins: the genetic modification might not be directed at the production of novel proteins, protein expression levels might be too low to be detected, and proteins might only be expressed in certain parts of a plant or during certain stages of development (Jasbeer *et al.*, 2008).

PROTEIN-BASED GMO DETECTION
Detection and quantification of GMO material via the newly introduced proteins.

A further limitation for **protein-based GMO detection** is the susceptibility of proteins to heat denaturation and to chemical, enzymatic or mechanical degradation. Since protein detection requires intact, correctly folded protein molecules, it is only possible to reliably detect proteins in raw, non-processed commodities (Miraglia *et al.*, 2004).

Protein-based methods rely on a specific binding between the protein of interest and an antibody against that protein. The antibody recognizes the protein molecule, binds to it, and the resulting complex can be detected, for example by a chromogenic (colour) reaction. This type of assay is referred to as immunoassay, since antibodies are the molecules that are produced during an immune reaction to recognize and eliminate foreign (pathogenic) molecules. The main technique applying this procedure is called ELISA (enzyme-linked immunosorbent assay, Figure 7.4). The antibody required to detect the protein can only be developed with prior access to the purified protein; the protein can be purified from the GMO itself, or it can be synthesized in a laboratory if the composition of the protein is known in detail. Immunoassays can be applied both for detection and quantification of protein, over a wide range of protein concentration. Such assays are available for many proteins that are expressed in commercially released GMOs (Michelini *et al.*, 2008).

ELISA
Acronym for Enzyme-linked Immunosorbent Assay. Method to detect and quantify specific proteins.

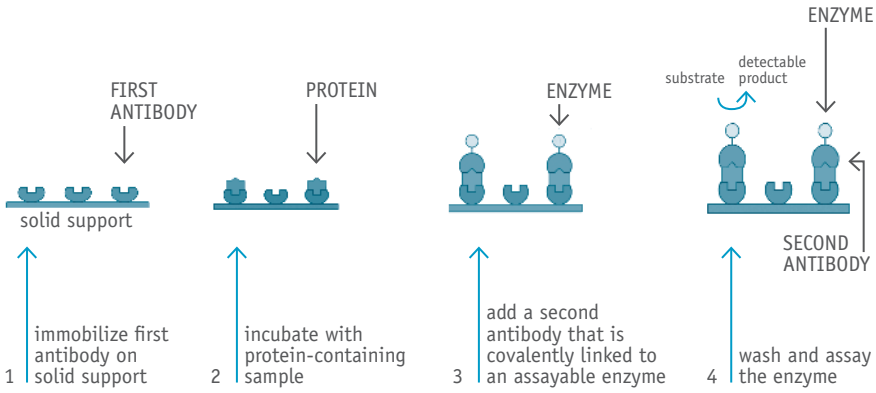
7.6.1 Enzyme-linked immunosorbent assay (ELISA)

In **ELISA**, a protein-antibody reaction takes place in solution on a solid support (plastic plates) and a protein-antibody complex is formed. This complex is usually

visualized by adding a second antibody that binds to the first antibody, and that is linked to a certain enzyme. This enzyme can catalyse the reaction of a specific substrate, which is added to the solution, to a coloured product (chromogenic detection). The intensity of the colour can be measured photometrically and used for quantitative assessments of protein concentration. ELISAs are available for several frequently engineered proteins in GM plants, including neomycin phosphotransferase (nptII), 5-enolpyruvyl-shikimate 3-phosphate synthase (EPSPS), the Bt insecticide Cry1Ab and phosphinotricin acetyltransferase (PAT) (Jasbeer *et al.*, 2008).

Figure 7.4 | **Enzyme-linked Immunosorbent Assay**

In this case a Sandwich-ELISA is depicted. A first antibody is immobilized on a solid support, followed by incubation with the target-protein containing solution. After a washing step (not shown), the second antibody, coupled to an assayable enzyme, is added and binds to the immobilized target protein. Finally the amount of bound secondary antibody, and thus target protein, can be assayed using the attached enzyme, which is usually done colorimetrically. In an easier approach, the target protein can be immobilized directly onto the plate, without a primary antibody.



Some ELISA plates are supplied with a calibration of known concentration of target protein in solution and a negative control defined by the absence of the target. These standards will exhibit distinctively different intensities of a given colour at the different concentrations of target molecules provided. By comparing the intensity of colour of the sample tested for GMO target molecules with that of the standards, it is possible to work out the concentration range of the target. These immunoassay measurements are semi-quantitative. Quantitative measurements can, however, be obtained by using a microplate reader which measures the absorbance of all samples and standards at the same time. This results in a very high precision of data acquisition and subsequently a precise calculation of target protein concentration in the test samples.

A major advantage of ELISA is the high specificity of the protein-antibody recognition, which allows accurate identification of proteins. Furthermore, they are fast, require only low work input, can be performed automatically to a large extent, and require only small investments in equipment and personnel.

However, ELISA may be around 100 times less sensitive than DNA-based methods, although detection of 0.01 percent of GM material has been described (Grothaus *et al.*, 2006). Furthermore, initial development and validation of a test for a specific protein is more time-consuming, and the supply of antibodies, which are derived from laboratory animals, is a limiting factor (Jasbeer *et al.*, 2008). Furthermore, protein detection and antibody affinity might be affected by the individual matrix under examination (Anklam *et al.*, 2002).

7.6.2 Lateral flow devices and dip sticks

LATERAL FLOW DEVICE
Rapid, on-site method for detecting GMO-derived protein, based on the same principle as ELISA.

Lateral flow devices and dip sticks are variations of the technology that ELISAs are based on; paper strips or plastic paddles on which antibody is captured on specific zones are used to detect protein targets derived from GMOs. The strip is dipped

into vials containing solutions of the sample to be tested. Each dip is followed by rinsing; the positive reaction is a colour change in a specific zone on the stick. Recent improvements of the dip stick have produced lateral flow strips in which reagents are transported through nylon membranes by capillary action. Antibodies specific to the target protein are coupled to a coloured reagent and are incorporated into the lateral flow strip. When the strip is brought into contact with a small amount of the sample containing the target protein, an antibody-antigen complex is formed with some of the antibody. The membrane contains two capture zones, one for the bound protein and the other for the coloured reagent. A coloured band appears in the capture zone corresponding to the bound antibody-protein complex and coloured reagent. Appearance of a single coloured band in the membrane is a negative test for the presence of the protein targeted. The presence of two bands represents detection of the target (Grothaus *et al.*, 2006).

These tests are available as kits and do not require major equipment or training, and thus represent a rapid GMO testing possibility. Sample preparation only involves homogenization of the sample and mixing with the reagents contained in the kit (Jasbeer *et al.*, 2008).

7.7 MOLECULAR DETECTION AND QUANTIFICATION OF GMOS – OTHER METHODS

Several other methods for the detection and quantification of GMOs have been proposed or are in developmental stages. Some of them are presented below – however, the main approved technologies for GMO analysis are PCR-based techniques and ELISA.

7.7.1 Chromatography and near infrared spectroscopy

If the chemical composition of a GMO has been altered, for example fatty acid or triglyceride content, chemical methods based on chromatography or near infrared

spectroscopy may be applied to detect these changes. These methods will detect differences in the chemical profile between GM organisms and conventional organisms. The applicability of such approaches has been demonstrated by investigating the triglyceride pattern of oils derived from GM canola by high performance liquid chromatography (HPLC). Triglyceride patterns and content can be compared between GM and non-GM samples. However, it should be noted that such techniques are only applicable when significant changes occur in the biochemical composition of GM plants or derived products. In addition, such methodologies only offer qualitative detection and no quantification (Anklam *et al.*, 2002). In particular, the addition of GM-derived products or raw material in small quantities to a larger lot of conventional material are probably not detectable given the sensitivity of the methods currently used.

7.7.2 Microarrays

MICROARRAYS
Comparatively new technology to detect GMOs. Possible to detect thousands of short DNA sequences in a single experimental setup.

Microarray technology (DNA-chip technology) has been developed in recent years for automated rapid screening of gene expression profiles and sequence variation of large numbers of samples. Microarray technology is based on the DNA hybridization principle, with the main difference that many (up to thousands) specific probes are attached to a solid surface and can be simultaneously detected. Different formats have been developed, including macroarrays, microarrays, high-density oligonucleotide arrays (gene chips or DNA chips) and microelectronic arrays.

GMO chip kits are designed to detect species-specific DNA of plants and viruses, frequently used transgene construction elements and specifically introduced genetic modifications, and thus allow the identification of approved and non-approved GMO varieties. One example of a GMO chip version that has been designed and tested for its applicability is capable of detecting species-specific DNA from soybean, maize, oilseed rape, rice, CaMV and several GMOs, including RR-soybean, Maximizer Bt 176 maize, Bt11 maize, Yieldgard Mon810 maize and Bt-Xtra maize. In addition,

GMO chips allow the detection of all GMOs that contain the widely used CaMV 35S promoter, Nos-terminator, *nptII*, *bar*, and *pat* genes (Leimanis *et al.*, 2006). Microarrays, in general, thus allow the detection, identification and quantification of a variety of GMOs in a single experimental setup.

7.8 SUMMARY OF GMO ANALYSIS: LIMITS AND OUTLOOK

As stated in the introduction, the field of GMO detection has a high relevance for all involved parties: research and development, producers, traders, consumers and legislation. Further progress in sampling and detection techniques and in traceability strategies needs to be made to enable adequate implementation and maintenance of GMO-relevant legislation and labelling requirements (Miraglia *et al.*, 2004). Promotion and implementation of reliable, international traceability strategies and agreements may also increase public trust in the transparency of GMOs and related products.

7.8.1 Summary of DNA and protein-based techniques

To summarize the previous sections, DNA and protein-based methods are currently the techniques of choice for GMO analysis. A PCR analysis can take between one to ten days and costs range from 100 to 400 euros. In comparison, an on-site ELISA takes two to eight hours and costs approximately 10 Euros; ELISA-based dipsticks take a few minutes to complete and cost around 3 euros (Miraglia *et al.*, 2004).

DNA-based analysis offers several advantages, including:

- a wide range of applications, from initial GMO screening to event-specific detection;
- the genome is the same in all cells of an organism, i.e. every part of an organism can be analysed;
- relative quantification, as required for labelling legislation, is possible;

- DNA is comparatively stable and can be isolated from a wide range of raw and processed matrices;
- a very high sensitivity.

Disadvantages of DNA-based methods include:

- the need for trained staff to operate high-end equipment;
- expensive, time-consuming and relatively unsuitable for on-site testing;
- DNA may be removed or degraded by certain processing procedures; certain food ingredients possibly interfere with DNA amplification and detection;
- PCR is very susceptible to cross-contamination;
- if no detailed sequence information of a GMO is available, DNA-based analysis is not possible.

Protein-based analysis offers the following advantages:

- comparatively cheap and less skilled personnel required;
- cheaper and less sophisticated equipment needed;
- fast conductance;
- quantification is possible;
- comparatively robust and simple assay formats;
- suitable for batch analysis of samples;
- possible to conduct on-site tests.

The disadvantages of protein-based analysis include:

- inferior sensitivity compared to DNA-based methods;
- the development of antibodies is difficult, expensive and requires skilled staff and equipment;
- only samples containing intact protein, i.e. fresh material, can be analysed;
- not possible to distinguish different events that produce the same protein (i.e. less specific than DNA-based methods);

- protein expression levels in a GM organism may vary significantly in a temporal and spatial manner;
- no relative, but absolute quantification;
- expression levels of target proteins may be too low to be detectable;
- reactivity of the antibody may be affected by other matrix components.

Thus, a careful evaluation of the most suitable analysis technique for a certain product should be performed to ensure that potential GMO contents are reliably, reproducibly and with high sensitivity detected and quantified. The choice of the technique may depend on a variety of factors, including the purpose (exact quantification for labelling legislation versus a simple yes/no result, GMO monitoring), the need for laboratory or on-site testing, financial background (including availability of personnel and equipment), exact GMO identification or just stating general GMO presence, the speed of analysis, composition of the food matrix to be analysed, etc. At present, however, PCR-based methods are the most widely applied and validated for GMO analysis purposes.

ANNEX 1

GENES OF INTEREST TO AGRICULTURE

A1.1 INTRODUCTION

Transgenic crops with novel agronomic and quality traits are grown in many developed and developing countries. A recent analysis of the current application of transgenic crops and the development over the last decade is provided by the International Service for the Acquisition of Agri-Biotech Applications (James, 2008). For a detailed account on the nature and extent of utilization of the various GM crops, one can consult online databases such as AGBIOS (http://www.agbios.com/dbase.php). The AGBIOS Web site includes details of the transgenes, the scientific background underpinning the traits and information on environmental and food safety issues of a variety of GM plants. A recent publication by the European Commission Joint Research Centre provides information about GM crops that are in the pipeline and expected to be marketed in the short to medium term, i.e. up to 2015 (Stein and Rodriguez-Cerezo, 2009). The database established by the authors is also available online at http://ipts.jrc.ec.europa.eu/publications/pub.cfm?id=2199. By surveying information in these and similar databases it is possible to get information on the

genes that have been used for the generation of transgenic crops, how these crops are commercially used and which additional crops are in developmental stages, in field trials or awaiting approval for commercial release. Each GMO is assigned a *Unique Identifier*, i.e. a code that allows allows direct identification of the GMO (Commission Regulation EC 65/2004).

A1.2 HERBICIDE TOLERANCE GENES

Glyphosate herbicide tolerance

The genetically modified glyphosate resistant crops contain a gene encoding the enzyme EPSPS, obtained from a strain of the soil inhabiting bacterium *Agrobacterium tumefaciens*. The EPSPS enzyme is an important part of the shikimate biochemical pathway which is required to produce aromatic amino acids, which plants need to grow and survive. EPSPS is also constitutively present in plants, but the enzyme is inhibited by binding of glyphosate. Conventional plants treated with glyphosate cannot produce the aromatic amino acids and die, whereas EPSPS from *A. tumefaciens* does not bind glyphosate and allows plants to survive the otherwise lethal effects of the herbicide (Tan *et al.*, 2006; Gianessi, 2008).

Glufosinate ammonium herbicide tolerance

Glufosinate ammonium is the active ingredient in the PPT herbicides. Glufosinate chemically resembles the amino acid glutamate and functions by inhibiting the enzyme glutamate synthase, which converts glutamate to glutamine. Glutamine synthesis is also involved in the ammonia detoxification of glufosinate resulting in reduced glutamine levels and increases in ammonia concentration. Elevated levels of ammonia damage cell membranes and impair photosynthesis. Glufosinate tolerance is the result of introducing a gene encoding the enzyme phosphinothricin-acetyl transferase (PAT). The gene was originally obtained from the soil actinomycete *Streptomyces hygroscopiens*. The PAT enzyme catalyses detoxification of phosphinothricin by acetylation (Duke, 2005; Tan *et al.*, 2006).

Sulfonylurea herbicide tolerance

Sulfonyl urea herbicides, such as triasulfuron and metsulfuron-methyl, target the enzyme acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), thereby inhibiting the biosynthesis of the branched chain animo acids valine, leucine and isoleucine (Tan *et al.*, 2005). This results in accumulation of toxic levels of the intermediate product alpha-ketoglutarate. In addition to the native ALS gene, herbicide tolerant crops contain the ALS gene from a tolerant line of *Arabadopsis thaliana*. This variant ALS gene differs from the wild type by one nucleotide and the resulting ALS enzyme differs by one amino acid from the wild type ALS enzyme. Still, this is sufficient to confer resistance to these herbicides, and provides an impressive example for the complexity and sensitivity of genes and proteins and the effects of mutations.

Oxynil herbicide tolerance

Oxynil herbicides and bromoxynil are effective against broad leaf weeds. Transgenic herbicide resistant crops contain a copy of the *bxn* gene isolated from the bacterium *Klebsiella pneumoniae*. The gene encodes a nitrilase which hydrolyses oxynil herbicides to non-phytotoxic compounds (Duke, 2005).

GENE STACKING
The development of transgenic plants that contain several transgenes, e.g. resistances to several different herbicides.

A recent development in herbicide tolerance is the development of plants containing several tolerance genes, allowing cocktails of different herbicides to be used (Green *et al.*, 2008). This technology is referred to as trait or **gene stacking**. Ideally, it will become possible to introduce not only herbicide tolerance traits, but also traits conferring insect resistance or quality traits (Halpin, 2005). One possible approach to this end is the development of artificial plant minichromosomes, capable of encoding many different, complex genes and regulatory sequences (Yu *et al.*, 2007).

A1.3 RESISTANCE TO BIOTIC STRESSES

Among insect pests, Lepidoptera (moths and butterflies) represent a diverse and important group. Most insect-resistant transgenic crop varieties developed so far

target the control of Lepidoptera, predominantly using transgene cassettes, including toxin-producing cry-type genes obtained from strains of the soil bacterium Bt. The Bt proteins bind to specific sites on the gut lining in susceptible insects (de Maagd *et al.*, 1999). The binding disrupts midgut ion balance which eventually leads to paralysis, bacterial sepsis and death. Important to note is that the original Bt cry-genes have been extensively modified, for example by deleting spurious splicing signals and optimizing the GC content, to improve the expression level in plants. Many cry genes exist that confer resistance to insects other than Lepidoptera. In addition to Bt cry genes, protease inhibitors, neuropeptides and peptide hormones that control and regulate the physiological processes of several insect pests have become candidates for developing insect-resistant crops. Other biocontrol toxins currently studied are chitinases, lectins, alpha-amylase inhibitors, cystatin and cholesterol-oxidase and glucosidase inhibitors (Christou *et al.*, 2006; Ranjekar *et al.*, 2003).

Among disease-causing organisms, viruses have received a lot of attention concerning the development of transgenic crops. This has been possible since the discovery of pathogen-derived resistance, where the expression of a viral protein (e.g. coat protein, replicase, helicase enzyme, etc.) in a transgenic plant renders that plant resistant to the virus (Prins *et al.*, 2008). As a result many viral genes have been cloned and used to transform crops. Genes encoding chitinases and glucanases have been used to generate plants resistant to fungal and bacterial pathogens, respectively. Other strategies for conferring resistance to pathogens in transgenic crops include genes for phytoalexine production pathways which are involved in pathogen-induced infection and defence, and R genes (resistance genes) which have been identified as responsible for additional defence mechanisms in plants (Campbell *et al.*, 2002).

A1.4 TOLERANCE TO ABIOTIC STRESSES

So far there are no commercialized transgenic crops with resistance to abiotic stresses such as drought, heat, salinity and frost. One possible explanation is that

the underlying genetic networks are rather complex, i.e. so far it has not been possible to identify single genes that would confer tolerance to these factors. However, a number of approaches are being developed to tackle these stress factors in crops (Bathnagar-Mathur *et al.*, 2008).

A1.5 QUALITY TRAITS

Modified flower colour

Many flowers including carnations, roses, lilies, chrysanthemums, roses and gerberas, which are important in the global flower trade, do not produce the blue pigment delphinidin. Transgenic carnation lines with unique violet/mauve colour have been developed. The genes of interest here include structural and regulatory genes of the flavanoid biosynthetic pathway.

Delayed fruit ripening and increased shelf life

Genes encoding an enzyme which degrades 1-aminocyclopropane 1-carboxylic acid (ACC), an ethylene precursor, and those encoding polygalacturonase (PG) have been suppressed in some transgenic plants. Suppression is accomplished by inserting a truncated or anti-sense version of the gene. Reduced ACC activity results in delayed fruit ripening while decreased activity of PG results in a lower level of cell wall breakdown and hence delays fruit softening and rotting (Prasanna *et al.*, 2007).

Modification of oil composition

Oilseed rape and soybean have been modified to increase the content of oleic acid in particular. The modified oils are lower in unsaturated fats and have greater heat stability than oils from the corresponding unmodified crops. In unmodified crops the FAD2 gene encodes a desaturase enzyme that converts C18:1 (oleic acid) to C18:2 and C18:3 acids. In the modified crop a mutant FAD2 gene prevents expression of the active desaturase, resulting in the accumulation of oleic acid (Kinney *et al.*, 2002).

Modified vitamin and mineral profiles

Vitamins and minerals are essential components of the human diet and dietary deficiencies of these nutrients can have severe effects on health and development. In addition to fortification and supplementation strategies for alleviating these deficiencies, transgenic crops with elevated and bio-available vitamins and minerals are being developed (Davies, 2007). Here the strategy is to express the genes responsible for the production or accumulation of the concerned nutrient in the edible parts of the plant. Thus promoters and other control sequences that target the expression of the gene(s) of interest to the correct part of the plant are highly important. In order to improve vitamin A production in rice the genes encoding phytoene synthase and phytoene desaturase have been expressed in the endosperm, resulting in the variety known as "Golden Rice". To improve iron accumulation and bio-availability in rice, genes such as ferritin synthase from soy (Fe storage), metallothionein (cystein-rich storage protein, improves Fe absorption) and a heat stable phytase gene (degrades phytic acid which inhibits Fe absorption) have been expressed in the rice endosperm.

A1.6 TRANSGENIC PLANTS AS BIOREACTORS FOR BIOPHARMACEUTICALS AND VACCINES

The first trials for the production of human proteins in plants dates back to the early 1990s; however, only in recent years has the use of transgenic plants as bioreactors for the production of small-molecule drugs or pharmaceutical proteins increasingly gained importance (Twyman *et al.*, 2005). The use of transgenic plants as a production platform presents a viable alternative to conventional production of such compounds, such as extraction from natural sources, various cell culture techniques or the use of animal bioreactors. In particular, plant-derived vaccines and antibodies are considered as promising (Tiwari *et al.*, 2009). Trials for the development of plants expressing vaccines in their edible parts, thus allowing cost-effective production and delivery of a vaccine, are a particularly intriguing option (Floss *et al.*, 2007).

REFERENCES

Allison, LA. 2007. *Fundamental molecular biology*. 1st edition. Malden, (MA), Wiley-Blackwell.

Anklam, E., Gadani, F., Heinze Hans Pijnenburg, P. & Van Den Eede, G. 2002. Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. *Eur. Food Res. Technol*. 214:3–26.

Basrur, P.K. & King, W.A. 2005. Genetics then and now: breeding the best and biotechnology. *Rev. Sci. Tech*. 24(1): 31-49.

Bazer, F.W. & Spencer, T.E. 2005. Reproductive biology in the era of genomics biology. *Theriogenology* 64(3): 442-56.

Benítez, T., Rincón, A.M., Limón, M.C. & Codón, A.C. 2004. Biocontrol mechanisms of Trichoderma strains. *Int. Microbiol*. 7(4): 249-60.

Bhatnagar-Mathur, P., Vadez, V. & Sharma, K.K. 2008. Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant Cell. Rep*. 27(3): 411-24.

Brophy, B., Smolenski, G., Wheeler, T., Wells, D., L'Huillier, P. & Laible, G. 2003. Cloned transgenic cattle produce milk with higher levels of ß-casein and K-casein. *Nature Biotechnology* 21: 157– 62.

Bull, A.T., Ward, A.C. & Goodfellow, M. 2000. Search and discovery strategies for biotechnology: the paradigm shift. *Microbiol. Mol. Biol. Rev.* 64(3): 573-606.

Campbell, M.A., Fitzgerald, H.A. & Ronald, P.C. 2002. Engineering pathogen resistance in crop plants. *Transgenic Res*. 11(6): 599-613.

Cankar, K., Stebih, D., Dreo, T., Zel, J. & Gruden, K. 2006. Critical points of DNA quantification by real-time PCR--effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. *BMC Biotechnol*. 6:37.

CBD (Convention on Biological Diversity). 5 June 1992. Rio de Janeiro. United Nations.

Chatterjee S., Chattopadhyay, P., Roy, S. & Sen, S.K. 2008. Bioremediation: a tool for cleaning polluted environments. *Journal of Applied Biosciences* 11: 594–601.

Chatterjee S., Chattopadhyay, P., Roy, S. & Sen, S.K. 2008. Bioremediation: a tool for cleaning polluted environments. *Journal of Applied Biosciences* 11: 594–601.

Chauthaiwale, V.M., Therwath, A. & Deshpandei., V.V. 1992**.** Bacteriophage lambda as a cloning vector. *Microbiological Reviews* pp. 577-591.

Christon, P., Capell, T., Kohli, A., Gatehouse, J.A. & Gataehouse, A.M. 2006. Recent developments and future prospects in insect pest control in transgenic crops. *Trends Plant Sci*. 11(6): 302-8.

Chung, S.M., Vaidya, M. & Tzafira, T. 2006. Agrobacterium is not alone: gene transfer to plants by viruses and other bacteria. *Trends Plant Sci*. 11(1): 1-4.

Clive, J. 2008. Global Status of Commercialized Biotech/GM Crops: *ISAAA Brief* No 39. Ithaca, New York, ISAA.

Dahiya, N., Tewari, R. & Hoondal, G.S. 2006. Biotechnological aspects of chitinolytic enzymes: a review. *Appl. Microbiol. Biotechnol.* 71(6): 773-82.

Darbani, B., Eimanifar A., Stewart, C.N. Jr. & Camargo, W.N. 2007. Methods to produce marker-free transgenic plants. *Biotechnol. J.* 2(1): 83-90.

Davey, M.R., Anthony, P., Power, J.B. & Lowe, K.C. 2005. Plant protoplasts: Status and biotechnological perspectives. *Biotechnol. Adv.* 23(2): 131-71.

Davies, K.M. 2007. Genetic modification of plant metabolism for human health benefits. *Mutat. Res.* 622(1-2): 122-37.

de Maagd, R.A., Bosch, D. & Stiekema, W. 1999. Bacillus thuringiensis-toxin-mediated insect resistance in plants. *Trends Plant Sci.* 4(1): 9-13.

Dekkers, J.C. 2004. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. *J. Anim. Sci.* 82 E-Suppl: E313-328.

Demain, A.L. & Adrio, J.L. 2008. Contributions of microorganisms to industrial biology. *Mol. Biotechnol.* 38(1): 41-55.

Denning, C. & Priddle, H. 2003. New frontiers in gene targeting and cloning: success, application and challenges in domestic animals and human embryonic stem cells. *Reproduction* 126(1): 1-11.

Donovan, D.M., Kerr, D.E. & Wall, R.J. 2005. Engineering disease-resistant cattle. *Transgenic Res.* 14(5): 563-7.

Duke, S.O. 2005. Taking stock of herbicide-resistant crops ten years after introduction. *Pest Manag. Sci.* 61(3): 211-8.

EPA (Environmental Protection Agency). Factsheet 006474 on Agrabacterium radiobacter strain K1026. Available online at: http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006474.htm

Etherton, T.D. & Bauman, D.E. 1998. Biology of somatotropin in growth and lactation of domestic animals. *Physiol. Rev.* 78(3): 745-61.

FAO. 2001: Agricultural biotechnology for developing countries. Results of an electronic forum.

FAO. 2004. The state of food and agriculture. Agricultural biotechnology: meeting the needs of the poor?

FAO. 2005. Status of research and application of crop biotechnologies in developing countries. Preliminary assessment

FAO. 2006. The role of biotechnology in exploring and protecting agricultural genetic resources.

FAO. 2007. Marker-assisted selection: current status and future perspectives in crops, livestock, forestry and fish.

FDA (U.S. Food and Drug Administration). Press Release, 6 February 2009. Retrieved on 15.10.2009 at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm109074.htm

Floss, D.M., Falkenburg, D. & Conrad, U. 2007. Production of vaccines and therapeutic antibodies for veterinary applications in transgenic plants: an overview. *Transgenic Res.* 16(3): 315-32.

Fravel, D.R. 2005. Commercialisation and implementation of biocontrol. *Annu. Rev. Phytopathol.* 43: 337–59.

Galli, C., Duchi, R., Crotti, G., Turini, P., Ponderato, N., Colleoni, S., Lagutina, I. & Lazzari, G. 2003. Bovine embryo technologies. *Theriogenology* 59(2): 599-616.

Gavrilescu, M. & Chisti, Y. 2005**.** Biotechnology – a sustainable alternative for chemical industry. *Biotechnology Advances* 23: 471–499.

Gellisen, G. (Ed). 2005. Production of recombinant proteins. 1st edition. Weinheim, Wiley-VCH.

Gelvin, S.B. 2003**.** Agrobacterium-mediated plant transformation. The biology behind the gene-jockeying tool. *Microbiol. Mol. Biol. Rev.* 67(1): 16-37.

Gianessi, L.P. 2008. Economic impacts of glyphosate-resistant crops. *Pest Manag. Sci.* 64(4): 346-52

Giraldo, P. & Montoliu, L. 2001**.** Size matters: use of YACs, BACs and PACs in transgenic animals. *Transgenic Research* 10**:** 83–103.

Gomez-Gil, B., Roque, A., Turnbull, J.F. & Inglis, V. 1998. A review on the use of microorganisms as probiotics. *Rev. Latinoam. Microbiol.* 40(3-4): 166-72.

Gray, N.K. & Wickens, M. 1998. Control of translation initiation in animals. *Annu. Rev. Cell. Dev. Biol.* 4: 399-458.

Green, J.M., Hazel, C.B., Forney, D.R. & Pugh, L.M. 2008. New multiple-herbicide crop resistance and formulation technology to augment the utility of glyphosate. *Pest Manag. Sci.* 64(4): 332-9.

Griffiths, A.J.F., Wessler, S.R., Lewontin, R.C., & Carroll, S.B. 2007. Introduction to genetic analyses. 9th edition. Palgrave Macmillan.

Grothaus, G.D., Bandla, M., Currier, T., Giroux, R., Jenkins, G.R., Lipp, M., Shan, G., Stave, J.W. & Pantella, V. 2006. Immunoassay as an analytical tool in agricultural biotechnology. *J. AOAC Int.* 89(4): 913-28.

Gurr, S.J. & Rushton, P.J. 2005**.** Engineering plants with increased disease resistance: how are we going to express it? *Trends in Biotechnology* 23(6): 283-290.

Haefner, S., Knietsch, A., Scholten, E., Braun, J., Lohscheidt, M. & Zelder, O. 2005. Biotechnological production and applications of phytases. *Appl. Microbiol. Biotechnol.* 68(5): 588-97.

Halpin, C. 2005. Gene stacking in transgenic plants--the challenge for 21st century plant biotechnology. *Plant Biotechnol. J.* 3(2): 141-55.

Herrera, G., Snyman, S.J. Thomson, J.A. 1994. Construction of a bioinsecticidal strain of pseudomonas fluorescens active against the sugarcane borer, Eldana saccharina. *Appl. Environ. Microbiol.* 60(2): 682-690.

Heyman, Y. 2005. Nuclear transfer: a new tool for reproductive biotechnology in cattle. *Reprod. Nutr. Dev.* 45(3): 353-61.

Hodges, C.A. & Stice, S.L. 2003. Generation of bovine transgenics using somatic cell nuclear transfer. *Reprod. Biol. Endocrinol.* 1: 81.

Houdebine, L.M. 2009. Production of pharmaceutical proteins by transgenic animals. *Comp. Immunol. Microbiol. Infect Dis.* 32(2): 107-21.

Hughes, T.A. 2006**.** Regulation of gene expression by alternative untranslated region. *Trends in Genetics* 22(3): 199-122.

International human genome sequencing consortium. 2004. Finishing the euchromatic sequence of the human genome. *Nature* 431: 931-945.

ISO (International Organization for Standardization). 2006. EN/TS 21568:2006, Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Sampling strategies 2006; European Committee for Standardization, Brussels, Belgium

ISTA (International Seed Testing Agency). 2004. Handbook on Seed Sampling. 2nd Edition. ISTA Bulking and Sampling Committee. M. Kruse (ed.).

Jana, S. & Deb, J.K. 2005. Strategies for efficient production of heterologous proteins in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 67: 289–298.

Jasbeer, K., Ghazali, F.M., Cheah, Y.K. & Son, R. 2008. Application of DNA and Immunoassay Analytical Methods for GMO Testing in Agricultural Crops and Plant-Derived Products. *ASEAN Food Journal* 15(1): 1-25.

J**obling, M.A. & Gill, P.** 2004. Encoded evidence: DNA in forensic analysis. *Nat. Rev. Genet.* 10: 739-51.

Juven-Gershon, T., Hsu, J-Y., Theisen, J.W.M. & Kadonaga, J.T. 2008. The RNA polymerase II core promoter - the gateway to transcription. *Curr. Opin. Cell. Biol.* Author manuscript; available in PMC.

Kikkert, J.R., Vidal, J.R. & Reisch, B.I. 2005. Stable transformation of plant cells by particle bombardment/biolistics. *Methods Mol. Biol.* 286: 61-78.

Kind, A. & Schnieke, A. 2008. Animal pharming, two decades on. *Transgenic Res.* 2008 17(6): 1025-33.

Kinney, A.J., Cahoon, E.B. & Hitz, W.D. 2002. Manipulating desaturase activities in transgenic crop plants. *Biochem. Soc. Trans.* 30(Pt 6): 1099-103.

Kuroiwa, Y., Kasinathan, P., Choi, Y.J., Naeem, R., Tomizuka, K., Sullivan, E.J., Knott, J.G. Duteau, A., Goldsby, R.A., Osborne, B.A., Ishida, I. & Robl, J.M. 2002. Cloned transchromosomic calves producing human immunoglobulin. *Nat. Biotechnol.* 20(9): 889-94.

Kuroiwa, Y., Kasinathan, P., Matsushita, H., Sathivaselan, J., Sullivan, E.J., Kakitani, M., Tomizuka, K., Ishida, I. & Robl, J.M. 2004. Sequential targeting of the genes encoding immunoglobulin-mu and prion protein in cattle. *Nat. Genet.* 36(7): 775-80.

Lacroix, B., Li, J., Tzifira, T. & Citovsky, V. 2006. Will you let me use your nucleus? How Agrobacterium gets its T-DNA expressed in the host plant cell. *Can. J. Physiol. Pharmacol.* 84(3-4): 333-45.

Law, B.A. & Mulholland, F. 1991. The influence of biotechnological developments on cheese manufacture. *Biotechnol. Genet. Eng. Rev.* 9: 369-409.

Lawrence, S. 2007. Billion dollar babies – biotech drugs as blockbusters. *Nature Biotechnology* 25: 380-382.

Leimanis, S., Hernández, M., Fernández, S., Boyer, F., Burns, M., Bruderer, S., Glouden, T., Harris, N., Kaeppeli, O., Philipp, P., Pia, M., Puigdomènech, P., Vaitilingom, M., Bertheau, Y. & Remacle, J. 2006. A microarray-based detection system for genetically modified (GM) food ingredients. *Plant Mol. Biol.* 61(1-2): 123-39.

Lipp, M., Shillito, R., Giroux, R., Spiegelhalter,F., Charlton, S., Pinero, D. & Song, P. 2005. Polymerase chain reaction technology as analytical tool in agricultural biotechnology. *J. AOAC Int.* 88(1): 136-55.

Madan, M.L. 2005. Animal biotechnology: applications and economic implications in developing countries. *Rev. Sci. Tech.* 24(1): 127-39.

Mattick, J.S. 1994**.** Introns: evolution and function. *Current Opinion in Genetics and Development* 4: 823-831.

McEvoy, T.G., Alink, F.M., Moreira, V.C., Watt, R.G. & Powell, K.A. 2006. Embryo technologies and animal health - consequences for the animal following ovum pick-up, in vitro embryo production and somatic cell nuclear transfer. *Theriogenology*. 65(5): 926-42.

Meeusen, E.N., Walker, J., Peters, A., Pastoret, P.P. & Jungersen, G. 2007. Current status of veterinary vaccines. *Clin. Microbiol. Rev.* 20(3): 489-510.

Melo, E.O., Canavessi, A.M., Franco, M.M. & Rumpf, R. 2007. Animal trangenesis: state of the art and applications. *J. Appl. Genet.* 48(1): 47-61.

Michelini, E., Simoni, P., Cevenini, L., Mezzanotte, L. & Roda, A. 2008. New trends in bioanalytical tools for the detection of genetically modified organisms: an update. *Anal. Bioanal. Chem.* 392(3): 355-67.

Miki, B. & McHugh, S. 2004. Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *J. Biotechnol.* 107(3): 193-232.

Miller, F.P., Vandome, A.F., McBrewster, J. 2009. *Central dogma of molecular biology*: History of molecular biology, Primary structure, DNA replication, Transcription (genetics). Alphascript Publishing.

Miraglia, M., Berdal, K.G., Brera, C., Corbisier, P., Holst-Jensen, A., Kok, E.J., Marvin, H.J., Schimmel, H., Rentsch, J., van Rie, J.P. & Zagon, J. 2004. Detection and traceability of genetically modified organisms in the food production chain. *Food Chem. Toxicol.* 42(7): 1157-80.

Morange. M. & Cobb, M. 2000. A history of molecular biology. 1st edition. Cambridge (MA), Harvard University Press.

Nestler, E.J. & Hyman, S.E. 2002**.** Regulation of gene expression. *Neuropsychopharmacology: The Fifth Generation of Progress.* Edited by Kenneth L. Davis, Dennis Charney, Joseph T. Coyle & Charles Nemeroff. American College of Neuropsychopharmacology.

Newell, C.A. 2000. Plant transformation techniques: Development and Application. *Mol. Biotechnol.* 16(1): 53-65.

Niemann, H., Kues, W. & Carnwath, J.W. 2005. Transgenic farm animals: present and future. *Rev. Sci. Tech.* 24(1): 285-98.

Official Journal of the European Union. 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.

Okkema, P.G. & Krause, M. 2005. Transcriptional regulation. *WormBook*, ed. The *C.elegans* Research Community, WormBook.

Ovesna, J., Demnerova, K. & Pouchova, V. 2008. The detection of genetically modified organisms: an overview. *In* F. Toldrá (ed.), *Meat Biotechnology*. Springer Science+Business Media, LLC.

Padidam, M. 2003. Chemically regulated gene expression in plants. *Curr. Opin. Plant Biol.* 6(2): 169-77.

Palmiter, R.D., Brinster, R.L., Hammer, R.E., Trumbauer, M.E., Rosenfeld, M.G., Birnberg, N.C. & Evans, R.M. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* 300(5893): 611-5.

Park, F. 2007. Lentiviral vectors: are they the future of animal transgenesis? *Physiol. Genomics* 31(2): 159-73.

Park, S.J. & Cochran, J.R. 2009**.** Protein engineering and design. 1st Edition. Boca Raton (FL), CRC Press, Taylor and Francis Group.

Patrushev, L.I. & Minkevich, I.G. 2008. The problem of the eukaryotic genome size. *Biochemistry (Moscow)* 73(13): 1519-1552.

Pearson, H. 2006 Genetics: What is a gene? *Nature* 441: 398-401.

Plasmid genome database: http://www.genomics.ceh.ac.uk/plasmiddb/index.html

Prasanna, V., Prabha, T.N. & Tharanathan, R.N. 2007. Fruit ripening phenomena--an overview. *Crit. Rev. Food Sci. Nutr.* 47(1): 1-19.

Prins, M., Laimer, M., Noris, E., Schubert, J., Wassenegger, M. & Tepfer, M. 2008. Strategies for antiviral resistance in transgenic plants. *Mol. Plant Pathol.* 9(1): 73-83.

Ramakrishnan, V. 2002. Ribosome structure and the mechanisms of translation. Cell, 108: 557–572.

Ramsay, M. 1994. Yeast artificial chromosome cloning. *Mol. Biotechnol.* 1(2): 181-201.

Ranjekar, P.K., Patankar, A., Gupta, V., Bhatnagar, R., Bentur, J. & Ananda Kumar, P. 2003 genetic engineering of crop plants for insect resistance. *Current Science* 84(3).

Rath, D. & Johnston, L.A. 2008. Application and commercialization of flow cytometrically sex-sorted semen. *Reprod. Domest. Anim.* 43 Suppl 2: 338-46.

Rege, J.E.O. 1996. Biotechnology options for improving livestock production in developing countries, with special reference to sub-Saharan Africa. *In* S.H.B. Lebbie and E. Kagwini. *Small Ruminant Research and Development in Africa. Proceedings of the Third Biennial Conference of the African Small Ruminant Research Network, UICC, Kampala, Uganda, 5-9 December 1994*. Nairobi, Kenya, ILRI (International Livestock Research Institute).

Remund, K.M., Dixon, D.A., Wright, D.L. & Holden, L.R. 2001. Statistical considerations in seed purity testing for transgenic traits. *Seed Science Research* 11:101–119. A tool for calculating sample plans, provided by ISTA, is available online at: http://www.seedtest.org/upload/cms/user/seedcalc6.zip.

Reznikoff, W.S. 1992**.** The lactose operon-controlling elements: a complex paradigm. *Molecular Microbiology* 6(17): 2419-2422.

Rizvi, P.Q., Choudhury, R.A. & Ali, A. 2009. Recent advances in biopesticides. *In* M.S. Khan, A. Zaidi & J. Musarrat (eds.), Microbial Strategies for Crop Improvement. Springer-Verlag Berling.

Robl, J.M., Kasinathan, P., Sullivan, E., Kuroiwa, Y., Tomizuka, K. & Ishida, J. 2003. Artificial chromosome vectors and expression of complex proteins in transgenic animals. *Theriogenology*. 59(1): 107-13.

Robl, J.M., Wang, Z., Kasisnathan, P. & Kuroiwa, Y. 2007. Transgenic animal production and animal biotechnology. *Theriogenology* 67(1): 127-33.

Rocha, J.L., Pomp, D. & Van Vleck, L.D. 2002. QTL analysis in livestock. *Methods Mol. Biol.* 195: 311-46.

Rogan, D. & Babiuk, L.A. 2005. Novel vaccines from biotechnology. *Rev. Sci. Tech.* 24(1): 159-74.

Roh, J.Y., Choi, J.Y., Li, M.S., Jin, B.R. & Je, Y.H. 2007. Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control. *J. Microbiol. Biotechnol.* 17(4): 547-59.

Rolinson, G.N. 1998. 40 years of beta-lactam research. *Journal of Antimicrobial Chemotherapy* 41: 589–603.

Ron, M. & Weller, J.I. 2007. From QTL to QTN identification in livestock-winning by points rather than knock-out: a review. *Anim. Genet.* 38(5): 429-39.

Schellenberg, M.J., Ritchie, D.B., & MacMillan., A.M. 2008. Pre-mRNA splicing: a complex picture in higher definition. *Trends in Biochemical Sciences* 33(6): 243-246.

Schmitt, B. & Henderson, L. 2005. Diagnostic tools for animal diseases. *Rev. Sci. Tech.* 24(1): 243-50.

Scupham, A.J., Bosworth, A.H., Ellis, W.R., Wacek, T.J., Albrecht, K.A. & Triplett, E.W. 1996. Inoculation with Sinorhizobium meliloti RMBPC-2 Increases Alfalfa Yield Compared with Inoculation with a Nonengineered Wild-Type Strain. *Appl. Environ/ Microbiol.* 62(11): 4260-4262.

Shewry, P.R., Jones, H.D. & Halford, N.G. 2008. Plant biotechnology: transgenic crops. *Adv. Biochem. Eng. Biotechnol.* 111: 149–186j.

Soccol, C.R., Vandenberghe, L.P., Woiciechowski, A.L., Thomaz-Soccol, V., Correia, C.T. & Pandey, A. 2003. Bioremediation: an important alternative for soil and industrial wastes clean-up. *Indian J. Exp. Biol.* 41(9): 1030-45.

Somers, D.A. & Makarevitch, I. 2004. Transgene integration in plants: poking or patching holes in promiscuous genomes? *Curr. Opin. Biotechnol.* (2): 126-31.

Stein, J.A. & Rodriguez-Cerezo, E. 2009. The global pipeline of new GM crops. Luxembourg, European Commission Joint Research Centre.

Stewart, C.N. Jr. 2005. Monitoring the presence and expression of transgenes in living plants. *Trends Plant Sci.* 10(8): 390-6.

Sullivan, E.J., Pommer, J. & Robl, J.M. 2008. Commercialising genetically engineered animal biomedical products. *Reprod. Fertil. Dev.* 20(1): 61-6.

Sullivan, E.J., Pommer. J. & Robl, J.M. 2008. Commercialising genetically engineered animal biomedical products., *Reprod, Fertil. Dev.* 20(1): 61-6.

Szewczyk, B., Hoyos-Carvajal, L., Paluszek, I., Skrzecz, I. & Lobo de Souza, M. 2006. Baculoviruses--re-emerging biopesticides. *Biotechnol. Adv.* 24(2): 143-60.

Tacket, C.O. 2009. Plant-based oral vaccines: results of human trials. *Curr. Top. Microbiol. Immunol.* 332: 103-17.

Tan, S., Evans, R. & Singh, B. 2006. Herbicidal inhibitors of amino acid biosynthesis and herbicide-tolerant crops. *Amino Acids* 30: 195–204.

Tan, S., Evans, R.R., Dahmer, M.L., Singh, B.K. & Shaner, D.L. 2005. Imidazolinone-tolerant crops: history, current status and future. *Pest Manag. Sci.* 61(3): 246-57.

Thomas, M.C. & Chiang, C-M. 2006. The general transcription machinery and general cofactors. *Crit. Rev. Biochem. Mol. Biol.* 41(3): 105-78.

Thorpe, T,A. 2007**.** History of plant tissue culture. *Mol. Biotecbnol.* 37(2): 169-80.

Tiwari, S., Verma, P.C., Singh, P.K. & Tuli, R. 2009. Plants as bioreactors for the production of vaccine antigens. *Biotechnol. Adv.* 27(4): 449-67.

Twyman, R.M., Schillberg, S. & Fischer, R. 2005. Transgenic plants in the biopharmaceutical market. *Expert Opin. Emerg. Drugs* 10(1): 185-218.

Vajta, G. & Gjerris, M. 2006. Science and technology of farm animal cloning: state of the art. *Anim. Reprod. Sci.* 92(3-4): 211-30.

Vicedo, B., Peñalver, R., Asins, M.J. & López, M.M. 1993. Biological Control of Agrobacterium *tumefaciens*, Colonization, and pAgK84 Transfer with Agrobacterium radiobacter K84 and the Tra Mutant Strain K1026. *Appl. Environ. Microbiol.* 59(1): 309-315.

Visel, A., Bristow, J. & Pennacchio, L.A. 2007. Enhancer identification through comparative genomics. *Semin. Cell. Dev. Biol*. 18(1): 140–152.

Voet, D. & Voet, J.G. 2004**.** *Biochemistry*. 3rd edition. Wiley & Sons.

Watson, J., Baker, T., Bell, S., Gann, A., Levine, M. & Losick, R. 2008. *Molecular biology of the gene*. Amsterdam, Addison-Wesley Longman.

Weimer, P.J. 1998. Manipulating ruminal fermentation: a microbial ecological perspective. *J. Anim. Sci.* 76(12): 3114-22.

Wheeler, M.B. 2007. Agricultural applications for transgenic livestock. *Trends Biotechnol.* 25(5): 204-10.

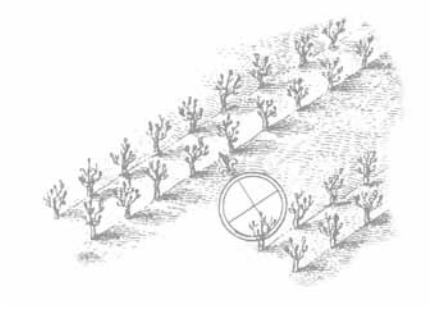
Wheeler, M.B., Walters, E.M. & Clark S.G. 2003. Transgenic animals in biomedicine and agriculture: outlook for the future. *Anim. Reprod. Sci.* 79(3-4): 265-89.

Whitelaw, C.B., Lillico, S.G. & King, T. 2008. Production of transgenic farm animals by viral vector-mediated gene transfer. *Reprod. Domest. Anim*. 43 Suppl 2: 355-8.

Williams, J.L 2005**.** The use of marker-assisted selection in animal breeding and biotechnology. *Rev. Sci. Tech*. 24(1): 379-91.

Yanofsky, C., Konan, K., Salsero, J. 1996. Some novel transcription attenuation mechanisms used by bacteria. *Biochemie* 78(11): 1017-1024(8).

Yu, W., Han, F. & Birchler, J.A. 2007. Engineered minichromosomes in plants. *Curr. Opin. Biotechnol.* 18(5): 425-31.



Design and layout: Pietro Bartoleschi and Arianna Guida (Studio Bartoleschi)
Cover illustrations elaborated from “l’Encyclopédie Diderot et d’Alembert”
Printed in Italy on ecological paper, Forest Stewardship Council (FSC) certified, May 2011

Biosafety Resource Book

MODULE A

MOLECULAR BIOLOGY AND GENETIC ENGINEERING

reviews the very basic scientific concepts and principles employed in producing GMOs, and provides a brief description of current and emerging uses of biotechnology in crops, livestock and fisheries.

For additional information please consult

www.fao.org/biotech

or contact

biotech-admin@fao.org

MODULE b

Biosafety Resource Book

ECOLOGICAL ASPECTS

FAO FIAT PANIS

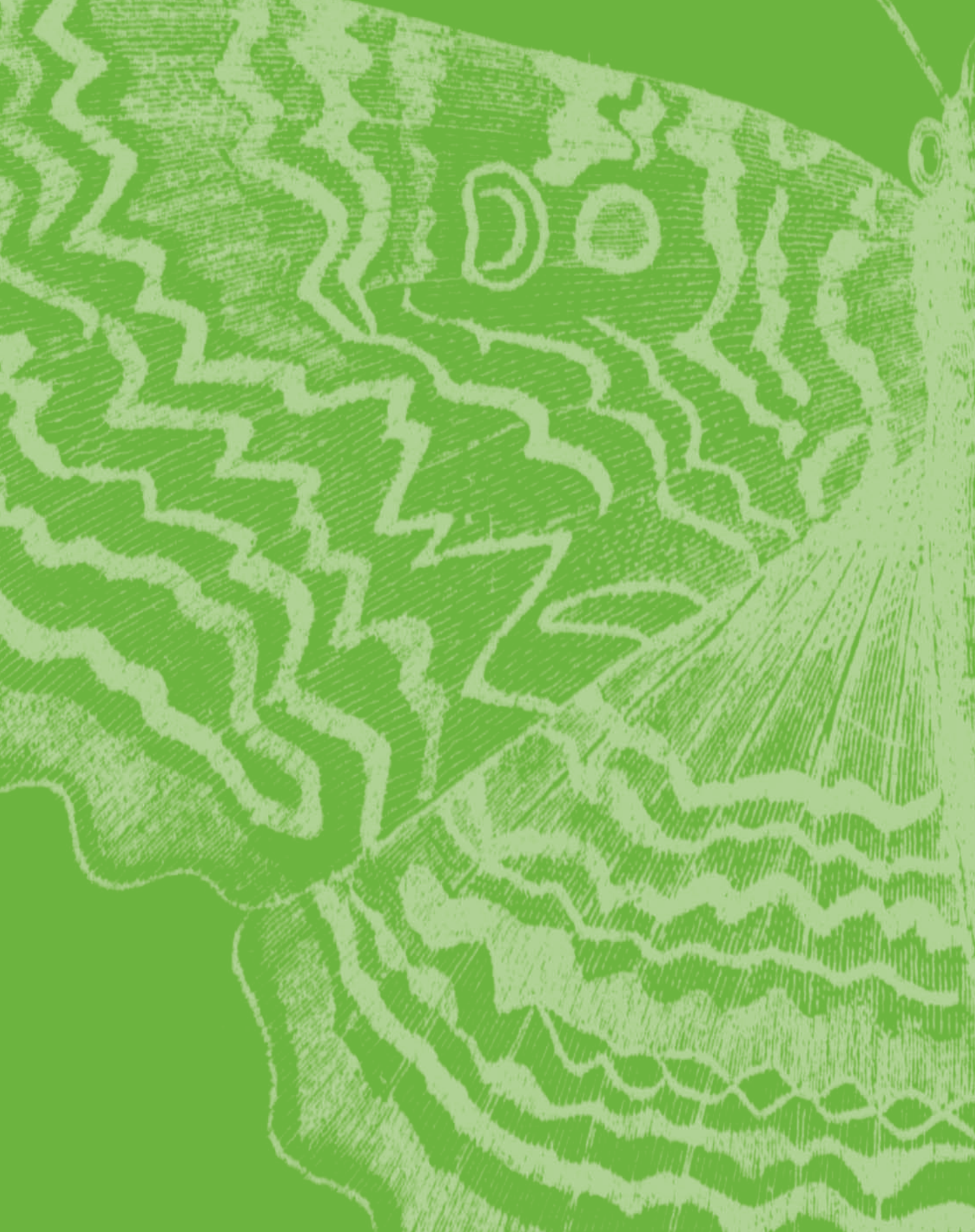
Biosafety Resource Book

MODULE b

ECOLOGICAL ASPECTS

Elizabeth Hodson de Jaramillo
Alessandra Sensi
Oliver Brandenberg
Kakoli Ghosh
Andrea Sonnino

Food and Agriculture
Organization of
the United Nations
Rome, 2011



LIST OF CONTRIBUTORS

This module has been prepared based on the lectures and contributions of:

Ervin Balazs
Department of Applied Genomics
Agricultural Research Institute of
the Hungarian Academy of Sciences
Martonvásár, Hungary

Oliver Brandenberg
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
Institute of Medical Virology,
University of Zürich,
Zürich, Switzerland)

Kakoli Ghosh
Plant Protection and Production Division
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

Elizabeth Hodson de Jaramillo
Departamento de Biología
Pontificia Universidad Javeriana
Bogotá, Colombia

Samuel Kiboi
School of Biological Sciences
University of Nairobi
Nairobi, Kenya

Ednah Kunjeku
Department of Plant Production
University of Venda
Thohoyandou, South Africa

Alessandra Sensi
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
EuropeAid Co-operation Office, Unit A.3
Centralised Operations for Europe,
the Mediterranean and the Middle East
Brussels, Belgium)

Andrea Sonnino
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

CONTENTS

MODULE b

LIST OF ABBREVIATIONS

AUIAB & NC	All-Union Institute of Applied Botany and New Crops
Bt	*Bacillus thuringiensis*
DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographical Information System
GM	genetically modified
GMOs	genetically modified organisms
GURT	genetic use restriction technology
HGT	horizontal gene transfer
IUCN	International Union for Conservation of Nature
MAS	marker assisted selection
NRC-CEI	National Reseach Council-Committee on Environmental Impacts
PGR	plant genetic resources
QTL	quantitative trait loci
UNCED	United Nations Conference on Environment and Development
VGT	vertical gene transfer

CHAPTER 1

INTRODUCTION TO ECOLOGY: BASIC CONCEPTS AND DEFINITIONS

1.1 WHAT IS ECOLOGY?

The word ecology, coined in 1866 by the German biologist Ernst Haeckel, derives from the Greek word "oikos" meaning "house" or "dwelling", and *logos* meaning "science" or "study". Thus, **ecology** is the "study of the household of nature", namely the systematic study of the distribution and abundance of living organisms - plants, animals, micro-organisms - and their interactions with one another and with their natural environment. The environment consists of both a living component, the **biotic environment** (organisms) and a non-living component, the **abiotic environment**, including physical factors such as temperature, sunlight, soil, rainfall, wind, and marine streams (Begon *et al.*, 2006).

ECOLOGY
The systematic study of the distribution and abundance of living organisms and their interactions with one another and with their natural environment.

BIOTIC ENVIRONMENT
All living components of the environment.

ABIOTIC ENVIRONMENT
All non-living environmental factors, e.g. temperature, rainfall, wind, insolation etc.

Few fields of study are more relevant to the human society and condition than the field in ecology. The increasing globalization of our economy and the resulting changes in social and political structures have a strong impact on our environment. One example is the both intentional and accidental dispersal of organisms, including pests and diseases, to all corners of the earth – ecological globalization on a grand scale. Generally, all activities of the human population affect the natural systems. Ecology, today, investigates several aspects and concerns:

- Interactions between organisms and the environment;
- How to understand, conserve, restore and sustainably use biodiversity;

- Impact of foreign species in ecosystems;
- Strategies for management, mitigation and reduction of impacts caused by human activity.

Critical considerations for ecological studies are that the natural world is diverse, complex and interconnected; that it is dynamic but at the same time stable and self-replenishing; that it is controlled by physical and biological processes, and that the order of nature is affected by human activity.

Life depends upon the abiotic, physical world, and vice versa affects it. Each organism continually exchanges materials and energy with the physical environment. Organisms interact with one another, directly or indirectly, through feeding relationships or *trophic interactions*. Trophic interactions involve biochemical transformations of energy and the transfer of energy from one individual to the next through the process of consumption. Materials move within ecosystems, and the pathways of such movements are closely associated with the flow of energy (Purves *et al.*, 2004). The flow of energy and its transfer efficiency characterize certain aspects of an ecosystem: the number of trophic levels, the relative importance of detritus, herbivore and predatory feeding, the steady-state values for biomass and accumulated detritus, and the turnover rates of organic matter in the community. Unlike energy, nutrients are retained within the ecosystem and are cycled between its abiotic and biotic components.

BIOGEOCHEMICAL CYCLES
The cycles by which an element or molecule moves through the biotic and abiotic compartments of Earth. Also referred to as nutrient cycles.

Human activities modify or disrupt the **global biogeochemical cycles** and create cycles of synthetic chemicals, such as pesticides. These changes can be large enough to cause serious environmental problems. However, ecosystems have the capacity to recover from many disturbances if the alterations have not been too large and the disturbing forces are reduced or eliminated. Controlling our manipulations of biogeochemical cycles so that ecosystems can continue to provide the goods and services upon which humanity depends is one of the major challenges facing modern societies (Purves *et al.*, 2004).

1.2 ORGANIZATION OF LIFE: HIERARCHY OF INTERACTIONS – LEVELS OF ECOLOGICAL ORGANIZATION

Below, in Table 1, are the most important terms and definitions listed that are employed in ecological sciences:

Table 1.1 | **Terms and definitions in Ecology**

Individual	An individual is a single organism inhabiting the environment as an isolated entity or as a member of a social group.
Species	Is the basic unit of classification of closely related organisms that have a high level of genetic similarity, are capable of interbreeding producing fertile offspring, and are reproductively isolated from other groups of organisms. This definition works well with animals. However, in some plant species fertile crossings can take place among related species.
Population	A population is a group of individuals of the same species living in a particular area. Populations are characterized by several parameters, such as abundance and distribution of their member organisms. The amount of resources available, diseases, competition for the limited resources, predation, birth and death rates, immigration and emigration affect the size of a population. Populations show characteristic age structures and age distributions. They are also characterized by an intrinsic rate of increase, the biotic potential. Populations do not show unlimited growth, they are limited by the carrying capacity of their habitat. Density-dependent and density-independent factors influence the size and growth of a population.
Community	A community is made up of the interacting, coexisting populations of different species occupying the same geographical area. Communities are characterized by the numbers of species present, their relative abundance, and their feeding and other ecological relationships. Within the community, there is competition for resources, various intra- and inter-species relationships and possibly exchange of genes. Populations and communities include only biotic factors, i.e. living components.
Ecosystem	An ecosystem is the complex of a living community (biotic factors) and abiotic factors (soil, rain, temperatures, etc.) in a given area. Ecosystems are further influenced by global phenomena such as climate patterns and nutrient cycles. The community influences the environment, and the environment influences the community, leading to changes and succession in the ecosystem. Energy flow, biogeochemical, water and nutrient cycles characterize ecosystems. The flow of energy is characterized by clearly defined trophic structures, biotic diversity, and material cycles (i.e. exchange of materials between living and non-living parts) within the ecosystem.

SPECIES
A group of organisms capable of interbreeding producing fertile offspring, and reproductively isolated from other groups of organisms.

POPULATION
A group of individuals of the same species living in a particular area.

ECOSYSTEM
The complex of a living community (biotic factors) and abiotic factors, and the interactions between them, in a given area.

Biosphere	The totality of ecosystems constitutes the biosphere, the portion of the earth that contains living species. It includes the atmosphere, oceans, soils and all the biogeochemical cycles that affect them.
Biome	Is another level of interaction placed between the ecosystem and the biosphere. A biome is a major ecological community or complex of communities, extending over a climatically and geographically defined area. There are two broad categories of biomes: aquatic and terrestrial. Biomes are defined by factors such as plant structures (e.g. trees, shrubs and grasses), leaf types (such as broadleaf and needleleaf), plant spacing (forest, woodland, savannah), and climate. Similar biomes exist on different continents and are often given local names. For example, a temperate grassland or shrubland biome is commonly known as *steppe* in central Asia, *prairie* in North America, and *pampas* in South America.

Attributes of individuals

The ecology of the individual is mainly concerned with the effects of the abiotic and biotic environment on its survival and reproduction rate. Any shortcomings in the phenotype or genotype of an individual will result in a selective pressure being exerted on it, and the individuals mostly affected by the adverse environmental factors will be removed from the population. Conversely, individuals with favourable traits will survive and show a higher rate of reproduction (see also chapter 3). This, of course, can be extended up to the species level, where such selection mechanisms determine the range of species in an ecosystem according to their environmental requirements and susceptibilities.

Attributes of populations

Populations have certain characteristics that define them. They have characteristic distributions over space, and they differ in age structure and size; they can be clumped together, randomly or uniformly distributed in their environment. They show growth rates (including negative growth) which define their abundance. The number of individuals in a population depends on the birth and death rates, and the net result of immigration and emigration. Each population has a tempo-spatial

structure, which includes features such as the density, spacing and movement of individuals, the proportion of individuals in different age classes, genetic variation, and the arrangement and size of areas of suitable habitat, all of which may vary in space and time. Population structure is also affected by the dynamics of parasites and their hosts, including for example, human diseases (Purves *et al.*, 2004). The structure of populations changes continually because demographic events, including births, deaths, immigration (movement of individuals into the area), and emigration (movement of individuals out of the area), are common occurrences. The study of birth, death and movement rates that give rise to population dynamics is known as **demography**. Individuals within a population compete with each other for resources such as space, mating partners and food. A population continues to grow until the habitat carrying capacity is reached. However, density independent factors, i.e. factors that do not depend on the actual number of individuals in a population, such as weather conditions (storms, floods, drought) and natural disasters (earthquakes, volcanic eruptions) may strongly influence population structure.

DEMOGRAPHY
The statistical study of populations, i.e. of the size, structure and distribution of populations, and spatial and/or temporal changes in them in response to birth, death and migration rates.

Genetic differentiation of populations depends far less on the movement of individuals among populations than on the forces of selection, mutation, and random change (genetic drift). Gene flow is the exchange of genetic information among populations resulting from the movement of individuals. The genetic structure of a population describes the distribution of the variation among individuals and among subpopulations, as well as the influence of mating systems on genetic variation. **Genetic variation** is important to a population because it is the basis of the population's capacity to become adapted to environmental change through evolution (Ricklefs and Miller, 1999). Genetic variation is also important to individuals: variation among an individual's progeny may increase the likelihood that at least some of them will be well adapted to particular habitat patches or to changing environmental conditions. Genetic variation is maintained primarily by random mutation and by gene flow from populations in other localities in which different genes have a selective advantage.

GENETIC VARIATION
Differences between individuals attributable to differences in the genotype.

Attributes of communities

COMMUNITIES
The entirety of interacting organisms/ populations sharing the same environment.

The same factors that define populations also define communities. **Communities** are usually defined by the interactions among the populations in the community and by the habitat in which the community occurs. Communities are characterized by several interrelated properties, grouped into the categories of *structure* and *function*. Structure refers to the number of species, called species richness, the types of species present and their relative abundances, the physical characteristics of the environment, and the trophic relationships among the interacting populations in the community. Rates of energy flow, properties of community resilience to perturbation, and net productivity are examples of community function. (Ricklefs and Miller, 1999). The species composition of ecological communities changes constantly over time.

Organisms interact with one another in different ways in their community:

COMPETITION
A contest between two or more organisms/ species for the same resource.

- Two organisms may mutually harm one another. This type of interaction – **competition** - is common when organisms use the same resource. Intraspecific competition is competition among individuals of the same species. Competition among species is referred to as interspecific competition.
- One organism may benefit itself while harming another, as when individuals of one species eat individuals of another (i.e. herbivores and carnivores). The eater is called a predator or parasite, and the eaten is called prey or host. These interactions are known as predator-prey or parasite-host interactions. Predators act as evolutionary agents by exerting selective pressure on their prey, which may eventually result in adaptation of the prey to protect itself against the predator (e.g. toxic hairs and bristles, tough spines, noxious chemicals and mimicry). This evolutionary mechanism also works in the other direction: once a prey has developed protective measures, selective pressure is exerted on the predator to develop features that allow it to overcome these restrictions and still be able to feed on the prey. It should be noted that these

are passive processes that cannot be influenced by the individual species/organism; please refer to Chapter 3 for details of evolution and speciation.

» Mutualistic interaction takes place when both participants benefit. **Mutualistic interactions** occur between members of different groups of organisms (between plants and prokaryotes, between fungi and protists, between animals and protists, between animals and plants and with other animals). If one participant benefits but the other is unaffected, the interaction is a commensalism. If one participant is harmed but the other is unaffected, the interaction is an amensalism. Mutualism, commensalism and amensalism are all different forms of symbiosis.

MUTUALISM
The close association of two different kinds of living organisms where there is benefit to both or where both receive an advantage from the association. A prominent example is the colonization of *Rhizobium* spp. inside the roots of leguminous plants.

Attributes of ecosystems

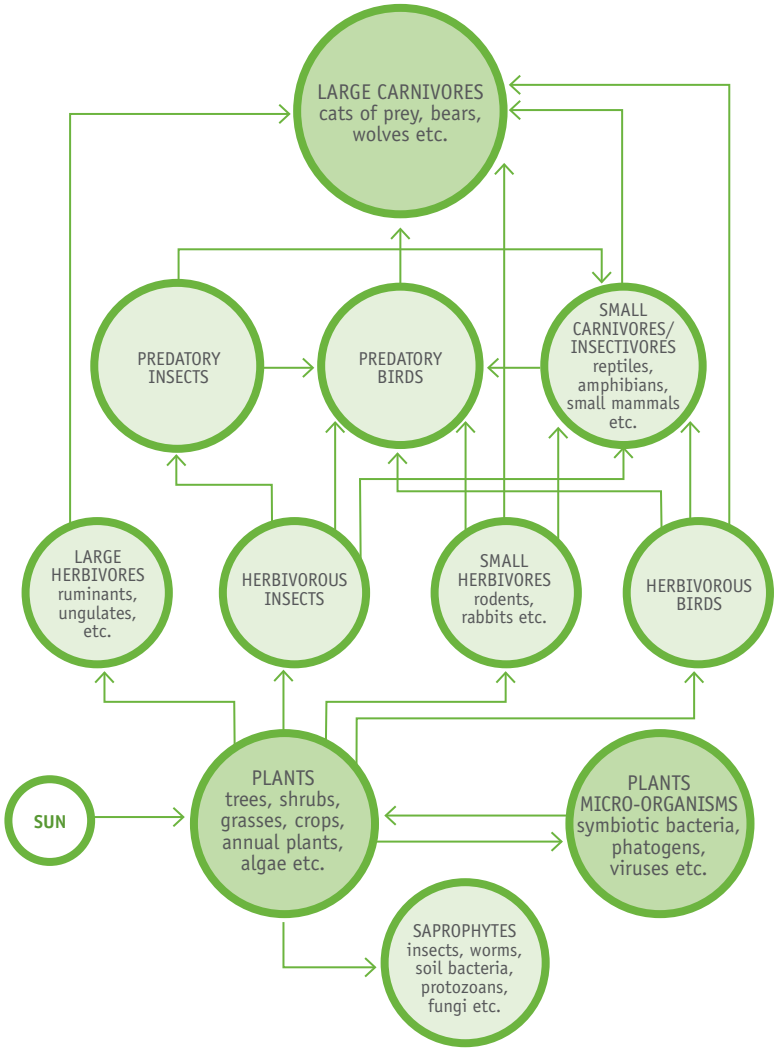
Ecosystems have **trophic levels**, which can be described as *energy pyramids* or *food pyramids*. The first trophic level is composed of primary producers that utilize light energy to produce high-energy compounds, usually in the form of carbohydrates (sugars). These organisms are referred to as autotrophs and are mostly plants (but include also bacteria and algae). Since only photosynthetic organisms are able to convert light energy to high-energy molecules, they have a key position in the ecosystem. Any factor that affects plants has strong implications on the ecosystem. The second trophic level is made up of primary consumers, which are the herbivores (i.e. plant-feeding organisms). The next level up is composed of secondary consumers, the carnivores (i.e. animal-feeding organisms); followed by top carnivores. Finally, all organisms that decompose dead organic matter are referred to as saprophytes, or detritivores. The feeding relationships ensure transfer of energy from one trophic level to the other. Importantly, only about 10 percent of energy is available for transfer from one trophic level to the next, which is why the possible number of carnivores and top carnivores is low, and hence trophic levels can be depicted as pyramids. The various interactions and feeding relationships between organisms in a community can also be depicted as food web (Box 1.1).

TROPHIC LEVELS
The different positions that an organism/species can occupy in the food chain.

BOX 1.1

TROPHIC LEVELS, FEEDING RELATIONSHIPS AND FOOD WEBS

Below, a highly simplified and generalized depiction of the various feeding relationships in a hypothetical "standard" ecosystem is shown.



As discussed in the text, all energy input in a typical ecosystem is derived from the sun.

Organisms that can directly utilize light energy and convert it into biomass are called autotrophs; this includes all photosynthetic plants. Plants and other autotrophic organisms therefore constitute the first trophic level, and are referred to as primary producers.

The primary producers are consumed by a large variety of primary consumers, the herbivores, constituting the second trophic level. The primary consumers are in turn consumed by secondary consumers (carnivores and insectivores) and so on. As already pointed out in the text, due to energy losses from one trophic level to the next, the number of trophic levels is limited (typically around five) and the maximum number of top carnivores is much lower compared with the number of primary producers. Please note that all organic matter, if not consumed by other organisms, is ultimately decomposed by the saprophytes (arrows between animals and saprophytes have been omitted for clarity).

In a detailed food web, individual species and their feeding relationships would be depicted, which can result in a highly complex diagram. Plants occupy a central position in food webs, being the primary source of energy and interacting with a large variety of organisms. Therefore, it would be helpful to carefully investigate the ecological relations and establish a detailed food web for a crop species that is subject to genetic modification. This would facilitate the prediction, investigation and assessment of the possible direct and indirect impacts of that genetic modification on the community interacting with and depending on that crop species.

CHAPTER 2

BIODIVERSITY: GENETICS, SPECIES AND ECOSYSTEMS

2.1 BIODIVERSITY

BIODIVERSITY
The variability among living organisms from all sources, including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part.

Biodiversity is the variation of life at all levels of biological organization; including genes, species, and ecosystems. At the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro in 1992, it was defined as: "The variability among living organisms from all sources, including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems". The three most commonly studied levels of biodiversity are ecosystem diversity, species diversity and genetic diversity. Given that the gene is the fundamental unit of natural selection, the real biodiversity is genetic diversity. For geneticists, biodiversity is the diversity of genes and organisms. They study processes such as mutations, gene exchanges and genome dynamics that occur at the deoxyribonucleic acid (DNA) level and are the driving force for evolution.

In ecological indexes, *Alpha* diversity refers to diversity within a particular area, community or ecosystem, and is measured by counting the number of taxa (usually species) within the ecosystem. *Beta* diversity is species diversity between

ecosystems; this involves comparing the number of taxa that are unique to each of the ecosystems in comparison. *Gamma* diversity is a measure of the overall diversity for different ecosystems within a region. Cultural or anthropological diversity is also involved when studying regional diversity. Biodiversity is not static; it is constantly changing. It is not evenly distributed on earth, and tends to be richer in the tropical regions. It varies with climate, altitude, soil composition and other physical parameters. Hotspots, regions with many endemic species, are usually found in areas with limited human impact, while regions with a high human population tend to have the lowest number of species, and thus a low biodiversity.

2.2 VALUES OF BIODIVERSITY

VALUES OF BIODIVERSITY
Include, among others, food supply and improvement, climate regulation, water purification, soil fertility, medicine, and aesthetic and cultural values.

Biodiversity has paramount importance for the social, cultural and economic development of humankind. Some ecosystem parameters that influence human society are air quality, climate (both global and local), water purification, disease control, biological pest control, pollination and prevention of erosion. Biodiversity plays a part in regulating the chemistry of our atmosphere and water supply; it is directly involved in water purification, recycling nutrients and providing fertile soils. There are a multitude of anthropocentric benefits of biodiversity in the areas of agriculture, science and medicine, industrial materials, ecological services, in leisure, and in cultural, aesthetic and intellectual value. The most direct and important use of biodiversity is as a source of food. Although a large number of plant species are edible, only a small percentage are used intensively in the production of food with significant nutritional value. Likewise, only a few of the numerous animal species are used for food production. Plant biodiversity is the basis of development and sustainability of agricultural production systems. A reduction in the genetic diversity of crops represents an increase in vulnerability to new pests and diseases. The economic value of the reservoir of genetic traits present in wild varieties and traditionally grown landraces is very high for improving crop

performance (The Academies of Sciences, 2007). Important crops, such as potato and coffee, are often derived from only a few genetic strains. Improvements in crop plants over the last 250 years have been largely due to harnessing the genetic diversity present in wild and domestic crop plants.

2.3 ECOSYSTEM, SPECIES, AND GENETIC DIVERSITY

Besides the large diversity of life on earth, our planet also contains a rich variety of habitats and ecosystems. Biodiversity is determined by both its biotic components, represented by living organisms, and its abiotic components, represented by the characteristics of the locations where the organisms live. In a strict sense, diversity is a measure of the heterogeneity of a system. This concept, when applied to biological systems, refers to the *biological heterogeneity that is defined as the amount and proportion of the different biological elements contained in a system*.

ECOSYSTEM DIVERSITY
Comprises the diversity of natural and artificial habitats, plus the species communities they contain.

Ecosystem diversity comprises the diversity of natural and artificial habitats, plus the species communities they contain. A certain species is usually found in a distinct ecological system, such as a tropical forest, a tropical savanna, or a coral reef. However, measuring ecosystem diversity may be difficult because the boundaries among communities and ecosystems are poorly defined. Human influence on natural ecosystems can result in severe consequences, for example, desertification and soil erosion, changes in the climate and the atmospheric composition, pest outbursts, and extinction of species.

SPECIES DIVERSITY
The number of species in an area and their relative abundance.

Species diversity is a result of the relation between the species' richness (number of species) and their relative abundance (number of individuals of each species) in a given area. A more precise concept is taxonomic diversity, which accounts for the diversity of a group of species that are more or less related. One of the major challenges for biologists today is to describe, classify and propose a sustainable

BOX 2.1

MEGADIVERSITY

The term megadiversity refers to areas which account for a high percentage of the world's biodiversity, by virtue of containing the most diverse and the highest number of plant and animal species.

Species diversity in natural habitats is higher in warm and rainy zones and decreases as latitude and altitude increase. The richest zones of the world in terms of biodiversity are undoubtedly the tropical rain forests, which cover 7 percent of the world's surface and contain 90 percent of the insect species of the world. (CBD, 2002; WCMC, 2002). Megadiverse countries are: Brazil, Colombia, Indonesia, China, Mexico, South Africa, Venezuela, Ecuador, United States, Papua New Guinea, India, Australia, Malaysia, Madagascar, Democratic Republic of the Congo, Philippines and Peru.

Concentrating on geographical areas and not on specific countries, 25 hotspots for biodiversity can be identified: Polynesia/Micronesia, Flower Province of California, Central America, Choco/Darien/ West Ecuador, central Chile, the Caribbean, Atlantic forest of Brazil, Brazilian Cerrado, forests of West Africa, the Karoo (succulents), the Mediterranean basin, Madagascar, the coastal forests of the eastern arc of Tanzania and Kenya, the Caucasus, Sri Lanka and the Western Ghats, south-central China, Sundaland, the Philippines, Wallacea, South West Australia, New Zealand and New Caledonia. These zones occupy only about 1.4 percent of the earth's surface and contain 44 percent of the known plants and 35 percent of the known animals. Tropical forests and Mediterranean zones predominate. Three of the zones are of special importance; Madagascar, the Philippines and Sundaland, followed by the Atlantic forests of Brazil and the Caribbean. The tropical Andes and Mediterranean basin are also important for their rich plant diversity.

use of organisms living in poorly understood habitats such as those in tropical rain forests, marine ecosystems and soil communities (Ricklefs and Miller, 1999). Species diversity has an important ecological effect on the structure of communities due to the interactions and interdependences among species: the reduction or disappearance of a given species may strongly influence other species that depend on it (WCMC, 2002).

GENETIC DIVERSITY
Refers to the total number of genetic varieties in the gene pool of a population, between populations or within a species, which are created, enhanced or maintained by evolutionary or selective forces.

Genetic diversity refers to the variation of genes within a certain population, among different populations or within a species. This type of diversity can be characterized at the molecular, population, species or ecosystem level. A lot of attention has been paid to genetic diversity due to its practical applications on plant and animal breeding and production, and for evolutionary studies (Purves *et al.*, 2004).

2.4 PROBLEMS AND THREATS TO BIODIVERSITY

Extinction has been a naturally occurring phenomenon over millions of years, without any human involvement. However, due to human activities and their effect on the environment, species and ecosystems have become increasingly threatened in an alarming way (WCMC, 2002), undermining the basis required for sustainable development. Almost all human activities result in a modification of natural environments. These modifications are harmful to the relative abundance of species and may even lead to species extinction. The main causes of environmental modification are: habitat alteration, for example by pollutants; habitat fragmentation, which can divide a big population into small isolated subpopulations and increase their risk of extinction if they are excessively reduced in size; habitat destruction, for example by converting forest to arable land or settlement areas; introduction of exotic or non-native species; overexploitation of plants and animals; soil, water and atmosphere pollution; alteration of the global climate; and agroindustries, including forestry. Although the loss of biodiversity in the form of crop varieties and domestic animal races has little

significance compared with global biodiversity, their **genetic erosion** is of immediate concern as it has profound implications and consequences for food supply and sustainability of local practices of animal and agricultural production (WCMC, 2002). Genetic erosion is difficult to assess quantitatively. It is usually calculated in an indirect way.

GENETIC EROSION
An already limited gene pool of an endangered species is further reduced when individuals from the endangered population die without breeding with other individuals form their population. Genetic variation, i.e. allelic diversity, is lost.

After 10 000 years of sedentary agriculture and the discovery of 50 000 varieties of edible plants, only 15 crop species represent today 90 percent of the food of the world. Rice, wheat and maize are the basic food for two thirds of the world's population. The continuous genetic erosion of wild species of cereals and other cultivated plants poses a risk for plant breeding programmes. Unless the loss of genetic diversity is controlled, by 2025 about 60 000 plant species - a quarter of the total world plant capital - might be lost (FNUAP, 2001). Fish stocks are also at risk. The Food and Agriculture Organization of the United Nations (FAO) estimates that 69 percent of marine commercial fish supplies of the world have been depleted. The greatest threats to biodiversity are destruction and deterioration of habitats, particularly in tropical developing countries (where biodiversity is concentrated), and the introduction of exotic species. Many of the factors affecting biodiversity are related to the needs of agricultural production: the increase in population and limited arable land have demanded increased agricultural productivity, and have led to more intensive agricultural practices, which have negative impacts on natural biodiversity. Habitat loss due to the expansion of human activities is identified as a main threat to 85 percent of all species described in the International Union for Conservation of Nature (IUCN) Red List. Main factors are urbanization and the increase in cultivated land surfaces (Amman *et al.*, 2003).

BOX 2.2

CAUSES OF BIODIVERSITY LOSS

CAUSES OF BIODIVERSITY LOSS
Include, among others, habitat conversion and fragmentation, genetic erosion, pollution, invasive species, unsuitable land use and management and natural events.

Biodiversity loss has been indicated by the loss in number of genetic resources and species. It has also been inferred from population decline and the degradation of ecosystem functions and processes. Several causes have been suggested; some of them are direct and others are identified as underlying factors.

Among the direct causes are:

- Habitat conversion/ fragmentation
- Unsuitable land use and management
- Domestication/genetic erosion
- Introduction of invasive and exotic species
- Trade
- Pollution
- Natural events

Among the underlying causes of biodiversity loss are:

- Demographic changes
- Poverty and inequality
- Climate change
- Public policies and markets
- Economic policies and structures

Climate change is also a factor of biodiversity loss. Excessive burning of fossil fuels is altering the balance of gases in the atmosphere; carbon dioxide (CO_2) is building up to high levels since more CO_2 is released than can be absorbed by the natural ecosystems. The interdependence of ecosystems is amply demonstrated here. For example, deforestation releases CO_2 and methane, which increases global temperatures. It also reduces ground cover, which disrupts water cycles as well as leading to soil erosion. The soil is washed into lakes and rivers, which silt and reduce aquatic biodiversity, among other effects.
Although deforestation can be controlled at a local level, the massive amount of deforestation is due to over-harvesting of trees for economic use, rather than local use. Most of the fossil fuel usage generating the CO_2 in the atmosphere stems from the industrialized nations and transition economies, but the effects are especially apparent throughout the less industrialized world.

2.5 COMMITMENTS AND OPPORTUNITIES

Biodiversity constitutes a part of the national patrimony of each country and represents great environmental, cultural and economic values. Conservation and sustainable use of biodiversity concerns all inhabitants of the world, represents an enormous potential for diverse countries and requires clearly defined strategies and policies for biodiversity management. As the human population grows, the demand for freshwater, food and energy resources puts the **sustainability** of the environment at risk. Developing adequate technologies and changing the way in which we use our resources are a growing challenge, and problems related to governability, social organization and human rights are of increasing importance in achieving sustainable results (FNUAP, 2001). In order to feed 8 000 million people that are expected to live on earth by 2025 and to improve their diets, and avoid malnutrition, the world's societies will have to improve food production and achieve a more equal distribution of food. Given that the available land suitable for agriculture is constantly being reduced, the increase in production will have to be achieved with higher yields instead of more cultivated surface. For example, scientists are working on genes that help plants to efficiently extract nutrients from soil, which would reduce the need for fertilizers; efforts are directed also at the development of drought resistant plants using the genes that allow certain species to survive drought (The Academies of Sciences, 2007). Development strategies that are beginning to materialize in several countries, especially in developing countries, are based mainly on a wide use of natural resources in a sustainable way, maximizing the potential of the plant sciences towards sustainable and environmentally responsible models of production for food, fuel and fibre, and incorporating them steadily into the agricultural sector. Biological resources represent a huge potential, insufficiently exploited, that requires strengthening and applying scientific and technological progress in order to understand, characterize and use these resources for the benefit of local communities. Biotechnology offers valuable tools to use these critical resources (Lemaux, 2008).

SUSTAINABILITY
The term describes how biological systems remain diverse and productive over time; in relation to humans, it refers to the potential for long-term maintenance of wellbeing, which in turn depends on the wellbeing of the environment and the responsible use of natural resources.

CHAPTER 3

EVOLUTION AND SPECIATION

3.1 THE DEVELOPMENT OF EVOLUTIONARY THEORY

EVOLUTION
The change in the inherited traits (i.e. the genetic material) of a population of organisms from one generation to the next. The process by which the present diversity of plant and animal life has arisen, and which continues to drive changes in form and mode of existence of all living organisms.

Patterns of reproduction, foraging, social interaction, growth and senescence and all other characteristics of an organism or species are shaped by natural selection through the interactions of organisms with their environment. The behavioral, physiological or developmental responses that allow an organism to accommodate or acclimate to the current conditions are called evolutionary adaptations. In biology, **evolution** is change in the inherited traits of a population of organisms from one generation to the next. These changes are caused by a combination of three processes: variation, reproduction and selection.

In a biosafety context, evolution is one of the most important concepts, considering the possible ecological/evolutionary impacts of escaped genetically modified organisms (GMOs). Some of the principal considerations in this context refer to natural selection pressures and genotype changes (which affect the rate of evolution), phenotypic variance, heritability, response to selection, inbreeding, outcrossing and genetic variation, among others.

Evolutionary biology became a defined science when Charles Darwin published "On the Origin of Species" in 1859. Darwin's Theory of Evolution is the widely

held notion that all life is related and has descended from a common ancestor. Darwin's general theory presumes that complex creatures evolve from more simplistic ancestors naturally over time. In a nutshell, as random genetic mutations occur within an organism's genetic code, the beneficial mutations are preserved because they aid survival, and ultimately reproduction, of the organism - a process known as "**natural selection**". These beneficial mutations are passed on to the next generation. Over time, beneficial mutations accumulate and the result is an entirely different organism (not just a variation of the original, but a distinct species that, in the case where the ancestor species still exists, is not capable of producing fertile offspring with the ancestor species). Natural selection acts to preserve and accumulate advantageous genetic mutations; natural selection in combination with reproduction is the preservation of a functional advantage that enables a species to compete better in the wild. Natural selection is the naturalistic equivalent to domestic breeding. Over the centuries, human breeders have produced dramatic changes in crop plant and domestic animal populations by selecting superior individuals for breeding; thus breeders eliminate undesirable traits gradually over time. Similarly, natural selection eliminates inferior species gradually over time, while new species constantly arise. However, there is of course no "goal" for evolution, as there is for breeders: instead, species are passively adapted in response to environmental influences.

NATURAL SELECTION
The process by which heritable traits that make it more likely for an organism to survive and successfully reproduce accumulate in a population over successive generations.

In Darwin's theory of natural selection, new (genetic) variants arise continually within populations. A small percentage of these variants cause their bearers to produce more offspring than others, because these variants confer some kind of advantage over other members of the population. These variants thrive and supplant their less productive competitors. The effect of numerous instances of such variation in combination with natural selection would lead to a species being modified over time (Purves *et al.*, 2004). Darwin did not know that the actual mode of inheritance was discovered in his lifetime. Gregor Mendel, in his experiments on hybrid peas, showed that genes from a mother and father organism do not blend.

POPULATION GENETICS
The study of the allele frequency distribution in a population and changes in allele frequency under the influence of natural selection, genetic drift, mutation and gene flow.

An offspring from a short and a tall parent may be medium sized, but it carries genes for shortness and tallness. The genes remain distinct and can be passed on to subsequent generations. It took a long time before Mendel's ideas were accepted. Mendel studied discrete traits; these traits did not vary continuously. The discrete genes Mendel discovered exist at defined frequencies in natural populations. Biologists wondered how and if these frequencies would change over time. Many scientists thought that the more common versions (alleles) of genes would increase in frequency simply because they were already present at high frequency. However, this is not necessarily true; the exact dynamics and frequencies of genes and their alleles, and the influence of environmental factors, are nowadays studied in the field of **population genetics**.

Population genetics investigates the evolutionary mechanisms of selection and genetic variability by developing quantitative predictions of changes in gene frequencies in response to selection. Hardy and Weinberg showed how genetic variation is retained in Mendelian inheritance, and that the frequency of an allele would not change over time simply due to the allele being rare or common. Their model assumed large populations in which there is random mating, no selection, no mutation, and no migration to or from the population. Later, R. A. Fisher showed that Mendel's Laws could explain continuous traits if the expression of these traits were due to the action of many genes (so-called polygenic traits). After this, geneticists accepted Mendel's Laws as the basic rules of genetics.

3.2 GENETIC BASIS OF THE EVOLUTIONARY MECHANISMS

Evolution, the change in the gene pool of a population over time, can occur in different ways. Two mechanisms remove alleles from a population: natural selection and genetic drift. Selection removes deleterious alleles from the gene pool by elimination of the organisms carrying the allele, while genetic drift is a random process that may result in removal of alleles from the gene pool. Three mechanisms

BOX 3.1

DEFINITIONS OF TERMS USED IN EVOLUTION BIOLOGY

Evolution is a change in the gene pool of a population over time. The process of evolution can be summarized in three key steps: genes mutate; individuals are selected; and populations evolve.

Gene is the unit of genetic inheritance that can be passed on from generation to generation. Usually, a gene is defined as a part of the DNA molecule that encodes a given gene product.

Genotype includes all genetic information of an organism and thus determines the structure and functioning of an organism.

Phenotype is the physical expression of the organism, resulting from the interaction of its genotype with the environment; the outward appearance of the organism.

Gene pool is the set of all genes/alleles in a species or population.

Allele is one of several alternative forms of a gene.

Locus is the location of a particular gene on a chromosome.

Mutation is a permanent change in the genotype (i.e. in DNA sequence) of an organism. Usually the term is applied to changes in genes resulting in new alleles.

Recombination refers to the mixing of genetic material via sexual reproduction.

Gene flow is the transfer of genes/alleles from one population to another.

add new alleles to the gene pool: mutation, recombination and gene flow. The amount of genetic variation found in a population is the balance between the actions of these mechanisms.

Mutation

MUTATION
Any permanent changes in the DNA sequence that make up an organism's genotype.

Mutations, permanent changes in the DNA sequences that make up an organism's genotype, range in size from a single DNA building block (DNA base) to a large segment of a chromosome, or even entire chromosome sets. There are many kinds of mutations. A point mutation is a mutation in which one "letter" (i.e. one base) of the genetic code is changed to another. Furthermore, lengths of DNA sequence can also be deleted from or inserted into a gene, and genes or parts of genes can become inverted or duplicated. Finally, mutations can take place at the level of chromosomes, leading to loss or addition of chromosome parts, entire chromosomes or even chromosome sets (polyploidy). Most mutations are thought to be neutral with regard to fitness of an organism. Mutations that result in amino acid substitutions can change the shape of a protein, potentially changing or eliminating its function. This can lead to inadequacies in biochemical pathways or interfere with the process of development. Only a very small percentage of mutations are beneficial (Purves *et al.*, 2004). A change in environment can cause previously neutral alleles to have selective advantages or disadvantages; in the short term evolution can run on "stored" variation in the gene pool of a population and thus is independent of the mutation rate.

RECOMBINATION
The production of a DNA molecule with segments derived from more than one parent DNA molecule. In eukaryotes, this is achieved by the reciprocal exchange of DNA between non-sister chromatids within an homologous pair of chromosomes during the first meiotic division.

Recombination

Genetic recombination is the process by which a strand of genetic material (DNA) is broken and then joined to a different DNA molecule in a controlled manner. In eukaryotes, recombination commonly occurs during meiosis as chromosomal crossover between paired chromosomes. Meiosis is a special type of cell division that occurs during formation of sperm and egg cells and gives them the correct number of chromosomes (i.e. a haploid set). Recombination can occur between different genes as well as within genes. Recombination within a gene can form a new allele. Recombination adds new alleles and combinations of alleles to the gene pool.

Gene flow (migration)

New individuals may enter a population by migration from another population. If they mate within the new population, they can introduce new alleles to the local gene pool. This process is called **gene flow**. Immigrants may add new alleles to the gene pool of the population, or may change the frequencies of alleles already present if they come from a population with different allele frequencies. Gene flow operates when there are no or only low spatial barriers that restrict movement of individuals between populations. Gene flow has strong relevance in the context of the introduction of GMOs into the environment, and is therefore the subject of specific attention in this module.

GENE FLOW
The transfer of genes or alleles from one population to another, e.g. by migration, resulting in addition of new alleles to the gene pool or changes in allele frequency.

Natural selection

Some individuals within a population produce more offspring than others. Over time, the frequency of the more prolific type will increase. The difference in reproductive capability is called natural selection. Natural selection is the only mechanism of adaptive evolution; it is defined as differential reproductive success of pre-existing classes of genetic variants in the gene pool. The most common action of natural selection is to remove unfit variants as they arise via mutation. This is called reproductive success, and is what is commonly referred to as "**survival of the fittest**". Fitness is a measure of reproductive success and is due to a number of selection factors:

SURVIVAL OF THE FITTEST
Synonym for natural selection, but not a correct scientific description.

- Survival/mortality selection. Any trait that promotes survival increases fitness.
- Sexual selection. Sexual selection is natural selection operating on factors that contribute to an organism's mating success. Traits that are a liability to survival can evolve when the reproductive success associated with a trait outweighs the liability incurred for survival. A male who lives a short time, but produces many offspring is much more successful than a long-lived one who produces few.
- Fecundity selection (size of offspring). High fecundity is due to the production of mature offspring resulting from earlier breeding or a higher number of fertilized eggs produced in species that provide little or no care for their young. The number of offspring gives family size, e.g. in species that take care of their young.

Genetic drift

GENETIC DRIFT
Change in allele frequency from one generation to another within a population, due to the sampling of finite numbers of genes that is inevitable in all finite-sized populations. The smaller the population, the greater is the genetic drift, with the result that some alleles are lost, and genetic diversity is reduced.

Allele frequencies can change randomly in a population. Genetic drift, more precisely termed allelic drift, is the process of change in the gene frequencies of a population due to chance events, which determine which alleles will be carried forward while others disappear. It is distinct from natural selection, a non-random process in which the tendency of alleles to become more or less widespread in a population over time is due to the alleles' effects on adaptive and reproductive success. When sampled from a population, the frequency of alleles differs slightly due only to chance. Alleles can increase or decrease in frequency due to genetic drift. A small percentage of alleles may continually change frequency in a single direction for several generations. A very few new mutant alleles can drift to fixation in this manner (Purves *et al.*, 2004). Both natural selection and genetic drift decrease genetic variation. If they were the only mechanisms of evolution, populations would eventually become homogeneous and further evolution would be impossible. There are, however, the three mechanisms that replace variation depleted by selection and drift, namely mutation, recombination and gene flow.

3.3 SPECIATION

SPECIATION
The evolutionary process by which new biological species arise.

Speciation is the evolutionary process by which new biological species arise. Speciation can take the form of a lineage-splitting event that produces two or more separate species from a common ancestor (cladogenesis), or evolution of a new species from an entire population without lineage split (anagenesis). There are various types of speciation: allopatric, peripatric, parapatric and sympatric speciation, which differ in geographical distribution and the mechanism of speciation of the populations in question. Separate species arise when accumulated genetic changes (mutations) between related populations no longer allow interbreeding, for instance after prolonged geographic separation (Ammann *et al.*, 2003).

The key to speciation is the evolution of genetic differences between the incipient

BOX 3.2

TYPES OF SPECIATION (PURVES *ET AL.*, 2004)

Allopatric *(allo=other, patric=place)* is thought to be the most common form of speciation. It occurs when a population is split into two (or more) geographically isolated subpopulations. In order for a speciation event to be considered allopatric, gene flow between the two subpopulations must be greatly reduced, and eventually the two populations' gene pools change independently until they can no longer interbreed, even if they were brought back together.

Peripatric *(peri=near);* new species are formed in isolated, small, peripheral populations which are prevented from exchanging genes with the main population. Genetic drift, and perhaps strong selective pressure, would cause rapid genetic change in the small population.

Parapatric *(para=beside);* the zones of two diverging populations are separate but overlap; there is no specific extrinsic barrier to gene flow. Individuals mate with their geographic neighbours more than with individuals in a different part of the population's range. In this mode, divergence may occur because of reduced gene flow within the population and varying selection pressure across the population's range.

Sympatric (sym=same) speciation occurs when two subpopulations become reproductively isolated without becoming geographically isolated in the first place. Insects that live on a single host plant provide a model for sympatric speciation. If a group of insects switched host plants they would not breed with other members of their species still living on their former host plant. The two subpopulations could diverge and speciate.

species. For a lineage to split once and for all, the two incipient species must have accumulated genetic differences that are expressed in a way that prevents mating between the two species, or causes mating to be unsuccessful (i.e. viable offspring is produced but cannot reproduce). These genetic differences need not be huge. A small change in the timing, location or rituals of mating could be enough. But still, some difference is necessary. This change might evolve by natural selection or genetic drift; reduced gene flow probably also plays a critical role in speciation. Speciation requires that the two incipient species are unable to produce viable/reproductive offspring together or that they avoid mating with members of the other group. Some of the barriers to gene flow (i.e. reproductive isolation) that may contribute to speciation are the evolution of different mating location, mating time or mating rituals; the lack of fit between sexual organs or offspring inviability or sterility. In terms of reproduction, plants have evolved various reproduction methods, in contrast to most animals. Many plants can reproduce sexually, by fertilizing other individuals or themselves, and asexually, by creating clones of themselves through vegetative reproduction, while most animals only reproduce sexually. Similarly, in terms of speciation, plants have more options than animals do. Two modes of speciation are particularly common in plants: speciation by hybridization or speciation by ploidy changes, i.e. changes in the number of chromosome sets per cell (Ricklefs and Miller, 1999).

All species, living and extinct, are believed to be descendants of a single ancestral species that lived more than 3.5 billion years ago (compared with an estimated age of the earth of 4.5 billion years). If speciation were a rare event, the biological world would be very different from what it is today. The result of speciation processes operating over billions of years is a world in which life is organized into millions of species, each adapted to live in a particular environment and to use environmental resources in a particular way (Purves *et al.*, 2004).

3.4 EXTINCTION

Extinction is a natural process in evolution that occurs when every living individual of a species disappears. The history of extinctions on earth includes several mass extinctions during which large numbers of species have disappeared in a rather short period of time (Purves *et al.*, 2004). The main causes of mass extinctions are major ecological disturbances such as volcanic eruptions, impacts of meteorites, fires, floods, species overexploitation, introduction of exotic or non-native species, habitat fragmentation, predation, parasitism, and a reduction of mutualism. Extinction depends on many ecological factors and characteristics of populations. Small populations are in higher danger of extinction than large populations, and **endemic species** - those which are limited to one or very few populations in specific locations and are not found anywhere else in the world - are at higher risk than widespread (cosmopolitan) species (Ricklefs and Miller, 1999). The rate of extinction is affected by population size, geographic range, age structure, and spatial distribution, and may result from a decrease in competitive ability.

EXTINCTION
The disappearance of every living member of a species.

ENDEMIC SPECIES
A species found only in a specific, unique geographic location.

Despite mass extinctions, speciation processes (new species arising from preexisting species) have allowed a net increment of species number throughout the history of life on earth. However, current concern arises due to the accelerated rates of extinction. During the past 400 years at least 350 vertebrate and 400 invertebrate species have gone extinct and several hundreds of plants have disappeared as a result of anthropogenic (human-caused) extinction. For the year 2000, the estimated risk of extinction for mammals was 24 percent and for birds, 12 percent. Several national and international conservationist agencies have developed strategies and programmes aimed at the conservation of wild species. For instance, the IUCN has created the Red List of species classifying them into categories according to the level or degree of threat: extinct, extinct in the wild, critical, endangered, vulnerable, susceptible, safe/low risk, insufficiently known and not evaluated. These categories are a guide to conservation activities and the priorities for action to rescue endangered species.

CHAPTER 4

AGRICULTURAL ECOLOGY – CENTRES OF ORIGIN/DIVERSITY

AGRO-ECOSYSTEM
A spatially and functionally coherent unit of agricultural activity, including the biotic and abiotic factors present in that unit and their interactions.

Agricultural activities have become the dominant ecological force over nearly one third of the land areas of the earth. **Agro-ecosystems** incorporate the concepts of ecology into their design and management. After a long history of separation and lack of interaction, ecologists and agronomists are now combining forces to study and help solve the problems confronting our food production systems, and to identify and mitigate the threats to natural resources and the ecological problems in agriculture. Development and application of this knowledge can lead to development of more sustainable agricultural ecosystems in harmony with their larger ecosystem and eco-region (NRCS, 2004). Agro-ecosystems are controlled, by definition, through the management of ecological processes.

For four million years, people procured food by hunting and gathering. Agriculture began in several settings more than 10 000 years ago, and was a necessary factor in the development of civilizations. Crops and farm animals were established by domestication and selection by farmers and breeders took place. Identifying the geographic origin of species is very useful, for example when plant breeders attempt to grow a crop in a zone with environmental conditions different from those of its original zone (Chrispeels and Sadava, 2003). Hybridization has played a major role in the development of new crops, in the modification of existing crops and in

the evolution of some troublesome weeds. One of the consequences of agriculture is the conversion of natural ecosystems into crop fields and pastures by removal of climax vegetation, controlling succession and exposing the soil to erosion.

4.1 DOMESTICATION OF SPECIES

DOMESTICATION
Human selection of plants/animals and subsequent development of a population that fits the needs of the farmer and consumer.

Domestication can be described as accelerated, goal-oriented evolution. Domestication implies changes in the genetic makeup and the morphological appearance and behaviour of plants and animals, so that they fit the needs of the farmer and consumer. For example, in wheat, as in many other grains, a major difference between the wild progenitor and domesticated descendants lies with seed dispersal. Wild plants spontaneously shed their seeds at maturity in order to assure their dispersal. Early farmers, during domestication, selected plants that held on to their seeds to minimize yield losses (Chrispeels and Sadava, 2003). Agriculture began at similar times in different regions of the world. In each of the regions, where the centres of origin of many crops are located, human populations domesticated different crops for similar uses. The evolution of crops is determined by three bottlenecks for genetic diversity: domestication, dispersal from the domestication centres, and crop improvement in the twentieth century. Early agricultural societies domesticated a few plant species, which served as the source of carbohydrates, proteins, fats and fibres. For instance, the emergence of Mediterranean and Middle Eastern civilizations was based on the domestication mainly of wheat, barley, lentils, peas, and linen. Later, the number of domesticated species increased and thus new crops appeared: oat, rye, olives, fruits, and others. Human migrations and exchanges among cultures helped to increase the number of plants cultivated in each region. The discovery of the American continent and all the exchanges by trade that came after led to a high level of genetic diversity within agricultural systems. Unfortunately, at the same time, the new available lands were increasingly used for extensive monocultures, especially of coffee, sugar cane, cotton and tobacco, in the colonies of the New World.

The domestication of plants and animals is based on the use of a reduced fraction of the existing biodiversity in each region and the adaptation of selected species to new environmental conditions suitable for human use. The domestication to new environmental conditions by **artificial selection** is opposed to the evolutionary mechanisms of adaptation by natural selection, as the environments where domestication takes place differ from the natural environments where wild relatives grow, and the selective pressures in each location are different. Domestication results in many morphological and physiological changes in plants or animals that make them, in general, easy to distinguish from their wild relatives. The most noticeable changes in plants are related to seed dispersal, seed dormancy, growth type, harvest index, photoperiod, organ size, presence of toxic compounds, and pest and disease resistance. Due to the fact that almost all crops share the same modified traits that distinguish them from their wild relatives, the whole set of new traits is known as domestication syndrome. Domestication is an artificial selection process directed by farmers. It leads to genetic changes and confers adaptive traits for environmental culture conditions, fitting farmers' and consumers' needs.

ARTIFICIAL SELECTION
Human selection and breeding of plants/animals for certain traits; opposed to natural selection.

4.2 CENTRES OF ORIGIN AND DIVERSIFICATION

Local and global geographic distribution of species depends on ecological conditions, both biotic and abiotic factors, and on evolutionary processes (Purves *et al*., 2004). The combination of all of these environmental conditions and processes determines the natural flora and fauna found in a given region, as well as the capability of developing certain crops in particular areas. The geographic distribution of wild relatives of a crop provides a general idea of where a crop may have originated. Careful botanic explorations are necessary to determine the precise distribution of wild progenitors. Additional genetic studies involving crosses between the crop and presumptive wild ancestors and a comparison of their genomes can identify in more detail a specific region of domestication.

The **centre of origin** is considered a geographical area where a group of organisms, either domesticated or wild, first developed its distinctive properties. Centres of origin of cultivated plants are identified by the number and diversity of wild species as well as the number of endemic species of the concerned *genus* in a given region, while the **centres of diversity** are recognized by the number and diversity of different varieties, wild and cultivated, of the concerned *species*. Centres of origin may simultaneously be centres of diversity. The centres of origin and centres of diversity of crop plants as known to us are largely based on circumstantial evidence. In the cases of crops that are extensively cultivated over wide geographical ranges, a large number of new varieties were continuously developed, involving a large number of parents, making the issues virtually intangible. For example, IR-64 rice appears to have had more than 100 parents, with consequent extensive genomic rearrangements, some natural and the others induced (Kameswara and Shantharam, 2004).

CENTRE OF ORIGIN
A geographical area where a group of organisms, either domesticated or wild, first developed its distinctive properties.

CENTRE OF DIVERSITY
A geographic area recognized on the basis of the present number and diversity of different varieties, wild and cultivated, of a species.

BOX 4.1

CENTRES OF ORIGIN AND CENTRES OF DIVERSITY

Centres of origin
The geographic locations where a particular domesticated plant species originated. These areas are the likeliest sources of natural genetic variation, and represent ideal targets for i*n situ* conservation.

Centres of diversity
The locations recognized on the basis of the number and diversity of different varieties, wild and cultivated, of a species.

The most important classification of the centres of origin of cultivated plants was established by the Russian geneticist Nikolai Ivanovich Vavilov (1887-1943). Vavilov realized the importance of genetic diversity of crops and their wild relatives for crop improvement.

His most important contribution was the identification of eight major geographic zones, known as "centres of diversity". There are a limited number of zones where crops originated. They are located in tropical and subtropical zones, at different elevations and a wide variety of topographies, and are characterized by distinct dry and wet seasons. They also correspond in many cases to the places where important human civilizations were established and flourished.

Centres of origin and domestication of cultivated species:

Based on the work of Vavilov in 1940 and Bryant in 2001

- **Chinese centre**:
  soybean (*Glycine max*),
  odder radish (*Raphanus sativus*),
  rapeseed (*Brassica rapa* var. *rapa*),
  pak-choi (*Brassica chinensis*),
  Chinese cabbage
  (*Brassica pekinensis*),
  Japanese shallot
  (*Allium fistulosum*),
  rakkyo (*Allium chinense*),
  cucumber (*Cucumis sativus*),
  yam (*Dioscorea batatas*),
  sorghum (*Sorghum* spp.),
  millet (*Panicoideae* subfamily).
- **Indo-Malayan centre**:
  Burma and Assam:
  egg plant (*Solanum melongena*),
  cucumber (*Cucumis sativus*),
  mung bean (*Phaseolus aureus*),
  cowpea (*Vigna sinensis*),
  taro (*Colocasia esculenta*),
  yam (*Dioscorea batatas*),
  rice (*Oryza sativa*).
- **Indochina and Malayan Archipelago**:
  banana (*Musa paradisiaca*),
  breadfruit (*Artocarpus altilis*),
  coconut (*Cocos nucifera*),
  sugarcane (*Saccharum* spp.).
- **Indo-Afghani-Central Asia centre**:
  garden pea (*Pisum sativum*),
  broad bean (*Vicia faba*),
  mung bean (*Phaseolus aureus*),
  leaf mustard (*Brassica juncea*),
  onion (*Allium cepa*),
  garlic (*Allium sativum*),
  spinach (*Spinacia oleracea*),
  carrot (*Daucus carota* var. *sativus*),
  apple (*Malus domestica*),
  chickpea (*Cicer arietinum*),
  lentil (*Lens culinaris*).

- **Near East centre**:
  lentil (*Lens culinaris*),
  lupin (*Lupinus albus*),
  barley (*Hordeum vulgare*),
  oat (*Avena sativa*),
  wheat (*Triticum* spp.).
- **Mediterranean centre**:
  celery (*Apium graveolens*),
  asparagus (*Asparagus officinalis*),
  beetroot (*Beta vulgaris* var. *crassa*),
  oilseed rape
  (*Brassica rapa* var. *rapa*),
  cabbage (*Brassica oleracea*
  var. *capitata*),
  parsnip (*Pastinaca sativa*), pea
  (*Pisum sativum*),
  rhubarb (*Rheum officinalis*), oat
  (*Avena sativa*),
  olive (*Olea europea*),
  wheat (*Triticum* spp.).
- **Abyssinian centre**:
  okra (*Abelmoschus esculentus*),
  watercress (*Lepidium sativum*),
  cowpea (*Vigna sinensis*),
  barley (*Hordeum vulgare*),
  coffee (*Coffea* spp.),
  sorghum (*Sorghum* spp.).
- **Mexico-Central America centre**:
  sweet pepper (*Capsicum* spp.),
  chili (*Capsicum annuum*),
  alcayota (*Cucurbita ficifolia*),
  pumpkin (*Cucurbita moschata*),
  sweet potato (*Ipomoea batatas*),
  lima bean (*Phaseolus lunatus*),
  kidney bean (*Phaseolus vulgaris*),
  maize (*Zea mays*),
  tomato (*Solanum lycopersicum*).
- **South American centre**:
  - Peru-Ecuador-Bolivia:
    sweet pepper(*Capsicum* spp.),
    chili (*Capsicum annuum*),
    pumpkin (*Cucurbita* spp.),
    tomato (*Solanum lycopersicum*),
    kidney bean (*Phaseolus vulgaris*),
    potato (*Solanum tuberosum*).
  - Chile:
    potato (*Solanum tuberosum*).
  - Brazil-Paraguay:
    peanut (*Arachis hypogaea*),
    cassava (*Manihot esculenta*).
- **North American centre**:
  sunflower (*Helianthus annuus*).
- **West African centre**:
  millet (*Panicoideae* subfamily),
  sorghum (*Sorghum* spp.).
- **North European centre**:
  oat (*Avena sativa*),
  rye (*Secale cereale*).

4.3 AGRO-ECOSYSTEM CHARACTERISTICS

Agricultural ecosystems - agro-ecosystems - have been described as domesticated ecosystems, in many ways intermediates between natural ecosystems (such as grasslands and forests) and fabricated ecosystems, such as cities (ASAP, 2004).

Just as natural ecosystems, they can be thought of as including the processes of primary production, consumption, and decomposition in interaction with abiotic environmental components and resulting in energy flow and nutrient cycling. Economic, social, and environmental factors must be added to this primary concept due to the human element that is so closely involved with agro-ecosystem creation and maintenance.

Any agro-ecosystem contains some or all of the following factors:

- Crops – plants cultivated for the benefit of humankind;
- Weeds – plants that are potential competitors to crops;
- Pests - animal predators and parasites;
- Pathogens – micro-organisms causing diseases;
- Domestic animals;
- Beneficial micro-organisms - e.g. rhizobia and other nitrogen fixing bacteria, mycorrhizal fungi;
- Beneficial arthropods - pollinators, natural enemies of pests;
- Soil.

Definitions of agro-ecosystems often include the entire support base of energy and material subsidies, seeds, chemicals, and even a social-political-economic matrix in which management decisions are made. Agro-ecosystems retain most, if not all, the functional properties of natural ecosystems — nutrient conservation mechanisms, energy storage and use patterns, and regulation of biotic diversity.

4.4 AGRO-ECOSYSTEM PATTERNS AND PROCESSES

Energy and matter flow in agro-ecosystems is altered greatly by human interference. Inputs are derived primarily from human sources and are often not self-sustaining. Agro-ecosystems are open systems where considerable energy is directed out of the system at the time of harvest, rather than stored in biomass that could accumulate within the system. In an agro-ecosystem, recycling of nutrients is minimal, and considerable quantities are lost with the harvest or as a result of leaching or erosion, due to a great reduction in permanent biomass levels held within the system. Because of the loss of niche diversity and a reduction in trophic interactions, populations within such a system are rarely self-regulating.

Agro-ecosystems are solar powered as are natural ecosystems, but differ from natural systems in the following points (ASAP, 2004):

- There are auxiliary energy sources that are used to enhance productivity; these sources are processed fuels along with animal and human labour as well as fertilizers;
- Biodiversity is notably reduced by human management in order to maximize yield of specific foodstuffs (plant or animal);
- Dominant plant and animal species are under artificial rather than natural selection; human inputs determine population sizes - linked to the productivity of the ecosystem.
- Control is external and goal-oriented rather than internal via subsystem feedback as in natural ecosystems.

Creation and maintenance of agro-ecosystems is necessarily concerned with the economic goals of productivity and conservation of the resource base. They are controlled, by definition, by management of ecological processes and they would not persist but for human intervention. It is for this reason that they are sometimes referred to as artificial systems as opposed to natural systems that do not require

intervention to persist through space and time. Knowledge of the ecological interactions occurring within an agro-ecosystem and the sustainable functioning of the system as a whole allows successful long-term management. Sustainability can be achieved by implementing an agriculture that is ecologically sound, resource-conserving and not environmentally degrading.

4.5 SUSTAINABLE AGRICULTURE

SUSTAINABLE AGRICULTURE
To maximize advantage of existing soil, nutrient and water cycles, energy flows, and soil organisms for food production, while at the same time the natural resource base upon which it depends is maintained.

Sustainable agriculture is both a philosophy and a system of farming. It has its roots in a set of values that reflect an awareness of both ecological and social realities. Sustainable agriculture systems are designed to maximize the advantage of existing soil, nutrient and water cycles, energy flows, and soil organisms for food production.

An ecologically sustainable agriculture maintains the natural resource base upon which it depends, relies on a minimum of artificial inputs from outside the farm system, manages pests through internal regulation mechanisms, and is able to recover form the disturbances caused by cultivation and harvest through successional processes minimizing waste and environmental damage, while maintaining or improving farm profitability (ASAP, 2004). In practice, such systems have tended to avoid the use of synthetic fertilizers, pesticides, growth regulators and livestock feed additives. Biological and cultural controls are used to manage pests, weeds and diseases.

Management of agro-ecosystems for sustainability both influences and is influenced by biodiversity. Sustainable practices leading to increased crop and genetic diversity have resulted in increased agro-ecosystem stability – for example, increasing crop diversity benefits agriculture by reducing insect pests. Conservation tillage increases habitat and wildlife diversity, and raises the numbers of beneficial insect species.

BOX 4.2

SUSTAINABLE AGRICULTURE (ASAP, 2004)

- is based on the prudent use of renewable and/or recyclable resources. It uses renewable energy sources such as biological, geothermal, hydroelectric, solar, or wind energy.
- protects the integrity of natural systems so that natural resources are continually regenerated. Sustainable agricultural systems should maintain or improve groundwater and surface water quality and regenerate healthy agricultural soils.
- improves the quality of life of individuals and communities. In order to stem the rural to urban migration, rural communities must offer people a good standard of living including diverse employment opportunities, health care, education, social services and cultural activities.
- is profitable. Transition to new ways of knowing, doing and being require incentives for all participants.
- is guided by a land ethic that considers the long-term well-being of all members of the land community. An agro-ecosystem should be viewed as a dynamic, interdependent community composed of soil, water, air and biotic species, with capacity for self-renewal.

Many of the approaches in conventional agriculture (minimum tillage, chemical banding) already indicate the way towards sustainable, efficient agriculture. Efforts to introduce safe products and practices (botanical pesticides, bio-control agents, imported manures, rock powders and mechanical weed control) are being pursued. Despite the reduced negative environmental damage associated with them, they remain problematic. Botanical pesticides also kill beneficial organisms, the release of bio-controls does not address the question of why pest outbreaks occur,

dependence on imported fertilizer materials makes the system vulnerable to supply disruptions, and excessive cultivation to control weeds is detrimental to the soil. As in conventional agricultural systems, the success of sustainable approaches is highly dependent on the skills and attitudes of the conductors (ASAP, 2004). What has become increasingly clear in the last few years is that good agronomy is based on an understanding of ecology. An agro-ecological approach is used increasingly by agricultural professionals to analyse the success of sustainable farming systems, and to identify ways of improving the productivity, profitability, and resource efficiency of these systems.

4.6 AGRICULTURAL BIODIVERSITY

AGRICULTURAL BIODIVERSITY Includes all components of biological diversity of relevance to food and agriculture, and all components of biological diversity that constitute the agricultural ecosystems.

Agricultural biodiversity is a broad term that includes all components of biological diversity of relevance to food and agriculture, and all components of biological diversity that constitute the agricultural ecosystems, also named agro-ecosystems: the variety and variability of animals, plants and micro-organisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agro-ecosystem, its structure and processes (CBD, COP decision V/5, Appendix). It includes crops and livestock and their wild relatives, but also many other organisms such as soil fauna, weeds, pests and predators. Agricultural biodiversity is the outcome of the interactions among genetic resources, the environment and the management systems and practices used by farmers. It is the result of both natural selection and human intervention developed over millennia.

GENETIC RESOURCES FOR FOOD AND AGRICULTURE Include the genetic resources of plants, animals and micro-organisms with relevance for food and agriculture.

Dimensions of agricultural biodiversity

1) **Genetic resources for food and agriculture,** which constitute the units of production in agriculture, and include cultivated and domesticated species, managed wild plants and animals, as well as wild relatives of cultivated and domesticated species:

- Plant genetic resources (PGR), including crops, wild plants harvested and managed for food, trees on farms, pasture and rangeland species;
- Animal genetic resources, including domesticated animals, wild animals hunted for food, wild and farmed fish and other aquatic organisms;
- Microbial and fungal genetic resources.

2) **Components of biodiversity that support ecosystem services** upon which agriculture is based. These include a diverse range of organisms that contribute, in varying degrees to, *inter alia*, nutrient cycling, pest and disease regulation, pollination, pollution and sediment regulation, maintenance of the hydrological cycle, erosion control, climate regulation and carbon sequestration.
3) **Abiotic factors**, such as local climatic and chemical factors and the physical structure and functioning of ecosystems, which have a determining effect on agricultural biodiversity.
4) **Socio-economic and cultural dimensions**. Agricultural biodiversity is largely shaped and maintained by human activities and management practices, and a large number of people depend on agricultural biodiversity for sustainable livelihoods. These dimensions include traditional and local knowledge of agricultural biodiversity, cultural factors and participatory processes, as well as tourism associated with agricultural landscapes.

Biodiversity and agriculture are strongly interrelated: while biodiversity is critical for agriculture, agriculture can also contribute to conservation and sustainable use of biodiversity. Indeed, sustainable agriculture both promotes and is enhanced by biodiversity. Maintenance of this biodiversity is essential for the sustainable production of food and other agricultural products and the benefits these provide to humanity, including food security, nutrition and livelihoods.

CHAPTER 5

CONSERVATION OF GENETIC RESOURCES

5.1 GENETIC RESOURCES FOR FOOD AND AGRICULTURE

GENETIC RESOURCES
The genetic material, in its overall diversity, of an agriculturally valuable plant or animal species.

Genetic resources for food and agriculture are the biological basis of world food security and, directly or indirectly, support the livelihoods of every person on earth. Considering their importance for both traditional farming and breeding as well as genetic engineering, they constitute a world patrimony of invaluable usefulness for human existence. PGR comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species (Fraleigh, 2006). Genetic diversity provides farmers and plant breeders with options to develop, through selection and breeding, new and more productive crops, resistant to virulent pests and diseases and adapted to changing environments.

Genetic diversity of the majority of modern crops is very limited in comparison with their wild ancestors. This reduction in diversity during crop evolution is not recent, as it began with crop domestication. The development of improved "*elite*" varieties during the twentieth century accelerated the pace of genetic erosion. The better performance and higher yield obtained with new varieties led farmers to stop using their local varieties and instead switch to high-yielding hybrids and new varieties preferred by consumers (Chrispeels and Sadava, 2003). Domestication,

artificial selection and constant manipulation of biological diversity by humankind over the past 10 000 years in conjunction with overall human activities impacting on the environment have resulted in a constant decline in genetic resources as well as the conversion of vast forest extensions, savannahs and prairies into cultivated land. Human societies, at present, are mostly applying monocultures, the worst condition with regard to diversity; two well known examples illustrating this are the devastating consequences of the Irish famine (caused by a potato disease) and the desertification of Sumer in ancient Mesopotamia due to soil salinization. Genetic erosion reduces considerably the possibilities for crop improvement for the world community and in particular the small farmers, who depend in many cases on wild species and natural habitats to subsist (Pullin, 2002).

FAO estimates that since 1900, 75 percent of crop genetic diversity has been lost. Without a constant contribution of new, "wild" genes, plant geneticists and breeders cannot continue improving basic crops. Plants obtained by means of crop selection must be invigorated every 5 to 15 years in order to provide them with new or better traits such as pest and disease resistance, higher yields, or higher tolerance to droughts and saline soils. The most effective way to achieve this is by mixing commercial varieties with wild ones. Many of the local varieties and wild species that are being lost may contain genes with potential utility to plant breeders and biotechnologists for crop improvement (FAO, 2001; WCMC, 2002).

The growing deterioration of natural and agricultural environments, and concerns for the loss of biodiversity, have resulted in rapid development of the discipline of conservation biology. The origins of genetic resource conservation and the interest of agriculturalists in the origin of domesticated crops and in the use of wild relatives for breeding programmes can be traced to the 1910s. By 1924 the Russian botanist Vavilov founded the All-Union Institute of Applied Botany and New Crops (AUIAB & NC) in Leningrad and established a large seed bank. The number and size of crop gene banks have continued to grow dramatically ever since.

5.2 CONSERVATION AND RESTORATION

CONSERVATION BIOLOGY deals with the use and management of the biodiversity present in natural and cultivated ecosystems in order to guarantee their renewal, conservation and productivity.

Conservation biology studies the use and management of the biodiversity present in natural and cultivated ecosystems in order to guarantee their renewal, conservation and productivity, thus providing benefits and opportunities for present and future generations. The main approaches used today in conservation biology include conservation strategies for undisturbed natural ecosystems, restoration strategies for disturbed ecosystems and sustainable use strategies for transformed ecosystems, which include agro-ecosystems, urban ecosystems, dams, gardens and recreation areas, among others. (WCMC, 2002).

RESTORATION ECOLOGY investigates how to recover and rehabilitate an ecosystem when degradation and decline are extreme.

When degradation and decline are extreme, and no preservation is possible, **restoration ecology** investigates how to recover and rehabilitate an ecosystem. Restoration involves species reintroduction, i.e. the total or partial replacement of extinct populations with the same or similar species having an ecological, social, cultural or economic value. The most effective way to conserve viable populations is to conserve zones which are large enough to allow species and their habitats to exist. An important concept in wildlife conservation is that of biological corridors, which are strips of land connecting fragmented habitats through which species can move from and to different fragments of their natural habitats. Corridors allow the recolonization of habitat fragments where populations have disappeared and help to avoid inbreeding or endogamy in small subpopulations (Pullin, 2002; Ricklefs and Miller, 1999). There is no global consensus on what constitutes an important species, but species may be singled out for conservation if they fall into one or more of the following categories:

i) threatened species,
ii) ecologically important species,
iii) species useful to humans, and
iv) species with non-use value.

5.3 *IN SITU* AND *EX SITU* CONSERVATION OF PLANT GENETIC RESOURCES

As already mentioned, agro-biodiversity is currently threatened by the progressive loss of plant genetic diversity. This problem has increased agriculture vulnerability and has also impoverished food provision for humans (Fraleigh, 2006). The growing concern on genetic erosion has led to the establishment of **germplasm** conservation programmes worldwide. The effort to save biodiversity is directed at both crops and wild relatives. Wild relatives of crops are critical for increasing and improving agricultural production by providing useful genes for resistance against disease and pests, abiotic stress tolerance (drought, salinity, water logging), as well as for improving nutritional qualities. They also provide ecosystem services such as pollination, nutrient recycling and water flow management. The effort to conserve crop wild relatives is taking place at national and global levels, as it is believed to be one of the most important ways to improve food security. Countries that are richest in genetic diversity are, generally, the poorest in economic terms, so that international efforts might be required to help them secure and conserve their genetic resources.

GERMPLASM
Can be defined as the sum of genetic resources for a given species. Germplasm can be conserved either by protecting organisms in their natural habitat (in situ), or by storing and preserving them in designated facilities (ex situ).

There are two complementary approaches for conservation of PGR, namely *in situ* and *ex situ*. *In situ* conservation involves maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields as components of traditional agricultural systems. *Ex situ* conservation involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration. Samples from such species are stored in centralized banks away from the origin. Approaches to *ex situ* conservation include methods such as seed storage banks, field gene banks, botanical gardens, world heritage sites, research centres and laboratories. DNA and pollen storage also contribute indirectly to *ex situ* conservation of PGR (Rao, 2004). Bioversity International and

the Svalbard Global Seed Vault efforts are directed at genetic resources conservation. As a part of the worldwide work, about 6 million accessions are being conserved by 1 300 seed banks around the world, although there has been limited success when using wild seeds in crop improvement crosses (Bryant, 2001).

5.3.1 *In situ* conservation of plant genetic resources

IN SITU CONSERVATION
Maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields.

The aim of *in situ* conservation is to protect habitats of target species so that a population of that species can stably persist and evolution processes are assured. It includes establishing protected areas such as national parks, caring for peasant plots containing local varieties, and preserving forests to protect medicinal or wild species used by indigenous communities. The vision is for the protected areas to allow for multiple uses, and to allow the systems to preserve rare, endangered and threatened species. In these systems, there is a need to increase the geographic distribution of target species, improve population structure, and influence the dynamics and genetic variability within and between populations. Further, identification of threats to target species in the wild and suitable mitigation actions are required, and effective management plans for ecosystems, genetic resources, ecological restoration and species recovery programmes need to be implemented. The *in situ* conservation strategies for natural ecosystems include national natural parks, forests, protected areas, reserves and sanctuaries, and, especially for agro-biodiversity, community and domestic parcels including landraces and folk varieties. *In situ* conservation of cultivated species is primarily concerned with the on-farm maintenance of traditional crop varieties (**landraces**) and with forage and agroforestry species (Rao, 2004). Active participation by farmers and other users of genetic resources is an important part of *in situ* conservation of cultivated species. Crop resources in landraces are passed from generation to generation of farmers and are subject to different selection pressures to fit specific farming situations.

LANDRACES
Traditional crop varieties, generally composed of a heterogeneous mixture of genotypes, adapted to local conditions and often providing valuable genes for crop improvement.

5.3.2 *Ex situ* conservation of plant genetic resources

Among the various *ex situ* conservation methods (germplasm banks), **seed storage** is the most convenient for long-term conservation of PGR. Seeds are dried to low moisture content and stored at subzero temperatures in cold stores or deep freezers. According to FAO, this technique accounts for 90 percent of the six million accessions conserved *ex situ* globally. One of the most important examples is the Svalbard Global Seed Vault, which is a secure seed bank located on the Norwegian island of Spitsbergen in the remote Arctic. The facility was established to preserve a wide variety of plant seeds from locations worldwide in an underground cavern, and holds duplicate samples, or "spare" copies, of seeds held in gene banks worldwide. The Seed Vault provides insurance against the loss of seeds in gene banks, as well as a refuge for seeds in the case of large-scale regional or global crises. However, there are a large number of important tropical and subtropical plant species which produce recalcitrant seeds that quickly lose viability and do not survive desiccation, hence conventional seed storage strategies are not possible. There are also a number of other important crop species that are sterile or do not easily produce seeds, or seed is highly heterozygous and clonal propagation is preferred to conserve elite genotypes.

SEED STORAGE
One of the most convenient methods for long-term conservation of plant genetic resources.

***Ex situ* conservation** requires skills in management of resources, development of infrastructure and facilities to accommodate the collections. It should be considered as a tool to ensure survival of wild populations and other diversity, and should be integrated into *in situ* conservation. The collections include:

- Whole plant/animal collections;
- Zoological parks and botanic gardens where species can be kept safe from threat;
- Wildlife research facilities;
- Germplasm collections of wild and domesticated taxa in any form including zygotes, gametes and somatic tissue.

EX SITU CONSERVATION
Conservation of genetic resources outside the native habitat, generally used to safeguard populations in danger of destruction, replacement or deterioration.

Strategies used in *ex situ* conservation include: seed banks and germplasm banks, reproduction propagation (as in clonal orchards) and reintroduction of endangered species into the wild.

5.4 BIOTECHNOLOGY FOR CHARACTERIZATION, CONSERVATION AND SUSTAINABLE USE OF BIODIVERSITY

Humans have manipulated the genetic make-up of plants and animals since agriculture began more than 10 000 years ago. This exploitation of the natural variation in biological organisms has given us the crops, plantation trees, farm animals and farmed fish of today, which often differ radically from their early ancestors. Increasing the efficiency of agricultural production can reduce these impacts; biotechnologies can have an important role in this respect. Biotechnology is an important tool for biodiversity conservation and utilization, and is a complement – not a substitute – for many areas of conventional agricultural research. It offers a range of tools to improve our understanding and management of genetic resources for food and agriculture. Modern biotechnologies can help to counteract trends of genetic erosion in all food and agriculture sectors (FAO, 2004). Molecular markers are one of the most valuable molecular biology techniques; they are used in identification and characterization of species, populations and genotypes, and are very useful for quantifying the genetic diversity within populations. Molecular **marker assisted selection** (MAS) is a powerful tool in conventional plant breeding and crop improvement programmes, because it facilitates the identification of genes with agronomic importance (pest and disease resistance genes), hybridization ratios, to distinguish variety lines, and enables the purity control and certification of varieties (Henry, 2000; FAO, 2007). Molecular techniques are also useful tools when studying the influence of plant genetic diversity on ecosystem sustainability, due to the fact that diversity within species may contribute in a significant way to the productivity of an agro-ecosystem. Modern agricultural biotechnology

MARKER-ASSISTED SELECTION (MAS)
A molecular biology technique used in breeding programmes to facilitate the identification and selection of genes with agronomic importance.

includes a range of tools that scientists employ to understand and manipulate the genetic make-up of organisms for use in the production or processing of agricultural products. Problems that are addressed include diseases and pests, abiotic stresses (drought, salinity), improving nutritional quality, creation of new diagnosis tools, measurement, conservation and study of genetic resources, and production of vaccines (FAO, 2004).

BOX 5.1

GERMPLASM CHARACTERIZATION

Germplasm characterization requires observation, measurement and documentation of heritable plant traits. There is need for identification, classification and confirmation of plant sample collections by using descriptors for the stored species:

- *Morphological descriptors*, which describe the morphology of the plant, which are easy and reliable to use and cheap, but are limited because of limited polymorphisms that can be visualized. They are also affected by the environment, which affects phenotypic expression. These descriptors can also be highly subjective.
- *Agronomic descriptors/ traits*, which are useful for crops, but require large-scale field experiments, and are labour-intensive.
- *Molecular descriptors*, which use molecular marker technology to identify polymorphisms. These descriptors have proved to be very useful in identifying and tracing genes of interest for use in plant breeding and genetic engineering. There is a high throughput of information and most techniques yield highly reproducible results.

GERMPLASM CHARACTERIZATION
Germplasm stored in seed banks needs to be characterized, e.g. according to morphological, agronomic and molecular descriptors.

Biotechnology is being utilized for collecting and storing genetic information through seed and tissue culture. It is also being used for detection and elimination of diseases in gene bank collections. Identification of desired genes using molecular techniques ensures that the genotypes of choice are used for downstream operations. Long-term storage using cryopreservation of tissue culture results in safer and more efficient storage and distribution of germplasm. Molecular techniques are used to confirm identities of germplasm when it is taken out of the banks for regeneration of plants, in addition to screening the accessions for identification of genes of interest.

The aim of modern breeders is the same as that of early farmers – to produce superior crops or animals. Conventional breeding, relying on the application of classic genetic principles based on the selection of phenotype or physical characteristics of the organism concerned, has been very successful in introducing desirable traits into crop cultivars or livestock breeds from domesticated or wild relatives or mutants. Biotechnology can make the application of conventional breeding methods more efficient (FAO, 2004). Progress in molecular techniques and *in vitro* culture of plant organs, tissues and cells has been increasing over the past 50 years. Traditional plant breeding combined with improved agricultural practices and modern biotechnology techniques have resulted in higher crop yields (Henry, 2000). Recombinant DNA technology has also been an important tool in crop improvement.

5.5 BIOTECHNOLOGY, BIODIVERSITY AND SUSTAINABLE AGRICULTURE

Biotechnology has the potential to improve sustainability in several ways and is expected, thereby, to help maintain natural as well as agricultural biodiversity. Agriculture has to respond, in addition to the traditional focus on higher yields, by addressing the protection of environmental goods as well as consumer concerns for food safety and quality. Biotechnology can overcome some production constraints

which are difficult or intractable to tackle by conventional methods. It can speed up conventional breeding programmes, create crops resistant to diseases and pests, reduce the use of toxic chemicals that harm the environment and human health, and it can provide diagnostic tools and vaccines that help control devastating human or animal diseases. It can improve the nutritional quality of staple foods such as rice and cassava and create new products for health and industrial uses (FAO, 2004).

Developing sustainable agricultural systems with minimal impact on biodiversity will require utilizing all available technologies while simultaneously encouraging appropriate farming practices. Biotechnology should be part of integrated and comprehensive agricultural research and development programmes that give priority to the problems of the poor. Biotechnology is not a substitute for research in other areas such as plant breeding, integrated pest and nutrient management and livestock breeding, and feeding and management systems (FAO, 2004). A great deal needs to be done so that developing country producers are empowered to make their own decisions regarding these technologies for their own benefit. Identifying small farmers' constraints to technology access and use continues to be an issue that the international community must address. Investments in biotechnology research capacity for the public sector will only be worthwhile if the current difficulties in delivering conventional technologies to subsistence farmers can be reversed (FAO, 2004). We need a better understanding of the sustainability of crop and animal production systems, as well as to promote the development of integrated crop management systems linked to biotechnology progress, in order to establish production systems more friendly to the environment and thus to guarantee resources to future generations.

BIOTECHNOLOGY, BIODIVERSITY AND SUSTAINABLE AGRICULTURE
Biotechnology has the potential to improve sustainability in several ways and is expected, thereby, to help maintain natural as well as agricultural biodiversity. Biotechnology should be part of integrated and comprehensive agricultural research and development programmes that give priority to the problems of the poor.

CHAPTER 6

GENE FLOW

GENE FLOW
The movement or exchange of genes between different species or between different populations of the same species.

Gene flow, also known as gene transfer, is the movement or exchange of genes between different species or between different populations of the same species. Genes may flow (transfer) from one organism to its offspring via reproduction with sexually compatible relatives, in which case it is called vertical gene transfer (VGT), or by other means – i.e. by infection - to totally unrelated species and families of organisms, generally referred to as horizontal gene transfer (HGT). Gene flow is a natural process, with importance for the maintenance of genetic variation in populations, as well as for the spread of new traits among populations and across species boundaries. It can add new alleles to the gene pool of populations or change the frequencies of alleles present (Ammann *et al.*, 2003). Gene transfer within species is almost essential to preserve the fitness of most species of plants and animals, naturally including many species of crop plants, and is the basis for evolution. In crops (and other flowering plants), gene flow typically involves movement of pollen and is dependent upon wind or animal vectors (pollinators). Gene flow occurs with all species, and thus with all crop species, but the amount of gene flow is a function of species biology. Given its importance, the processes that affect gene flow have been widely studied and generally are well understood.

6.1 VERTICAL GENE TRANSFER

Vertical gene flow occurs naturally between crops and weeds and from crop-to-crop. It occurs between sexually compatible plants and wild relatives if the appropriate conditions are met. Gene transfer between crops and sexually compatible relatives has occurred since the domestication of plants began more than 10 000 years ago. Over the centuries farmers kept seed from the best plants in their crops that had been formed by mutation or had arisen from natural crosses. Gradually, major differences arose between the domesticated and wild species, so that farmers were keeping plants that contained combinations of genes that improved the domestic attributes of the crops (Ammann *et al.*, 2003). Most ecological scientists agree that vertical gene flow between crops and wild species is not an environmental problem unless it leads to undesirable consequences. In nature, vertical gene flow takes place through pollen transfer to the ovaries. For plants, gene flow may occur by pollen spreading from one population to another. The pollen may be spread in a variety of ways, e.g. by wind, water or insects and other animals. In self-pollinating plants, pollen transfer can be simply by gravity. Genes from the resulting offspring can be spread further by pollen or by seeds.

VERTICAL GENE TRANSFER
Gene flow from a parent organism to offspring, during sexual or asexual reproduction.

VGT from GM crops to non-GM crops or wild relatives of the crop in question is regarded as one of the major problems associated with the cultivation and release of GM crops. The minimum requirements for gene flow from a GM plant to a non-GM plant to occur are the presence of a sexually-compatible non-GM population in close proximity to the GM population, the possibility of **outcrossing** between the two populations and the production of fertile hybrids. The degree of outcrossing varies amongst species: maize and millet, for example, are typically cross-pollinated while rice, wheat and barley are primarily self-pollinated. An important aspect is that gene flow refers to the exchange of genes among populations and not simply to the dispersal of pollen or seeds. **Introgression** is what defines the stable incorporation of genes from one pool to another, and determines the actual gene flow between

OUTCROSSING
The occurrence of crosses, and thus transfer of genetic material, between two distinct species/ populations.

INTROGRESSION
Movement of gene(s) between the gene pools of different species, typically by production of interspecific hybrids that backcross with one of the parent species.

populations. Introgression takes place by creation of a hybrid plant (i.e. a cross between a GMO variant and a wild relative) and subsequent backcrossing of that hybrid with the parent species, resulting in the introduction of foreign genes to the parent species gene pool.

Since vertical gene transfer can potentially happen between crops/wild relatives and GM crops as soon as the latter are introduced to an environment, it is important to know the crop progenitors as well as their wild relatives in order to assess the likelihood of gene transfer. The answer to the question "Does gene transfer occur?" now seems clear: gene flow is inevitable from those crops that naturally outcross both to conventional varieties of the same crop and to a small number of wild relatives, although this latter phenomenon is usually a rare event. However, for ecologists and agronomists the key question is "Does it matter?" More specifically, does outcrossing of transgenes affect fitness of recipient offspring in both natural and agricultural ecosystems? The inherent characteristics of a crop and its proximity to closely related plants are some of the factors that determine the likelihood of gene transfer to other plants. The key to understanding vertical gene flow is knowledge of the sexual compatibility of the crop with other species growing in the same habitat.

Factors affecting VGT

Gene flow depends on many ecological and agronomical factors: the reproductive biology of the plant species, whether or not the crop is allowed to flower, how far its pollen travels, success of fertilization, extent of seed dispersal and seed survival, among others. Even if a gene does "escape", its future may be bleak if it handicaps its new host. The probability of successful pollination depends on a great number of interrelated factors, including level of pollen production, rate of self- and cross-fertilization of receptor plants, rate of pollen dispersion, pollinating agents, spatial distance between donor and recipient population, local density of recipient population, and differences in phenology between crop

and wild population. There is, therefore, a need to evaluate crop and recipient populations' overlap in space and time, hybridization between different crops, the stable incorporation of the transgene into the population (introgression) depending on the fertility of the hybrid produced, and use of landraces. In Kenya, for example, farmers frequently cross landraces with the improved varieties, thus crop-to-crop gene flow is already widespread.

A trait with a selective advantage and improved fitness has a chance of accumulating in a population. If the trait is outcrossed with wild relatives, it has a good chance of accumulating in the wild population, and that trait may be preferentially attained. There must be a benefit associated with the given gene in order for it to persist. If there is such a benefit, for example by increasing survival or reproduction, it is likely to spread more rapidly through the population. Conversely, if it has a detrimental impact on the fitness of individuals, the rate of gene flow is likely to be reduced and the gene may eventually be lost.

BOX 6.1

KEY ISSUES TO CONSIDER FOR VERTICAL GENE FLOW IN CROPS

- Sexual compatibility between plant species, presence of wild or domesticated relatives
- Pollen production rate
- Outcrossing rate and auto-pollination
- Pollen dispersal rate
- Pollen viability and competitive ability
- Characteristics of the pollinating agents
- Spatial distances between GMOs and recipients
- Environmental factors
- Local density of the population
- Temporal differences in flowering (phenologic isolation). Synchrony of flowering (timing for pollen shed, anthesis) and receptivity must coincide for the crop and nearby relatives
- The resulting offspring must be viable and fertile

6.2 HORIZONTAL GENE TRANSFER

HORIZONTAL GENE TRANSFER An organism incorporates genetic material from another organism without being the offspring of that organism.

HGT refers to non-sexual gene transfer between totally unrelated species and families of organisms. HGT is not a new phenomenon: it has regularly occurred during the history of life on earth. It has been a very important feature in the evolution of species and will continue to be important, but there is no obvious reason why its rate should be enhanced or altered by biotechnology and GMOs. HGT is very common for bacteria, where DNA may move easily between unrelated bacterial species, but not so common between other groups of organisms. HGT is frequently an essential component of the pathogenic relationship between a host and a pathogenic micro-organism. For example, gene transfer from bacteria to plants is a well-known natural phenomenon and forms the basis for one method of plant genetic manipulation. The bacteria concerned, *Agrobacterium* species, have evolved a series of plasmid-borne genes that enable them to attach to exposed cells in wounded plants, transferring genes from the plasmid to apparently random sites within the plant genome (Chrispeels and Sadava, 2003). *Agrobacterium* genes are thus introduced into plant genomes and this process constitutes the basis for *Agrobacterium*-mediated plant genetic transformation. There is no evidence to date that other bacteria have evolved specific methods to transfer genes to plants or animals (Ammann *et al.*, 2003).

6.3 MECHANISMS AND EFFECTS OF HORIZONTAL AND VERTICAL GENE TRANSFER

As discussed, HGT between species is a common natural process, especially among micro-organisms. There is an absolute need for the incoming DNA to be integrated and replicated with the host genome if the genes carried are to be stably maintained in the new host. If foreign DNA that has entered a new host is to be maintained, there is a need for it to confer a selective advantage on the host. If it does not, the frequency at which these genes are present in a population will remain at

BOX 6.2

DIFFERENCES AND SIMILARITIES BETWEEN TRANSGENE INSERTION AND TRANSPOSITION

It has been argued that transgene insertion is not different from transposition, a natural process that involves genes moving from one locus to another in a genome. These so-called jumping genes, or mobile genetic elements, that are also used as vectors in genetic engineering, were first discovered in maize.

There are similarities between the two processes, but there are also major and fundamental differences. *Transposons occur endogenously in plants*, whereas *transgenes are artificially introduced*. Transposition is a rare event that seldom gives rise to new plant characteristics, while transgenic plants are common today and may display significantly altered characteristics compared with the parent organism. Both transgenes and transposons can silence genes and activate dormant genes. Both are capable of causing mutations. Activation of the transposase gene in plants is not a foreign process, and normally transposons do not provide new information in a plant.

The integration and movement of the transposons are regulated by the plant. There is conflicting evidence about the insertion of transposons: some studies show site-specific insertions, while others show no site preferences. In maize, the frequency of transposition depends on the developmental stage of the plant. Activation and deactivation of transposon genes are controlled by the plant. Transgenes, on the other hand, are present throughout the development of the plant. Transposons are also known to insert in sequences that have been duplicated before, although it is not clear whether this is a consequence of the jump or a presupposition of insertion.

The integration of transgenes is irreversible, while transposon insertion is reversible, although this reversibility might imply higher risks associated with transposition in terms of side effects because of the mutations they cause.

the frequency at which these genes are taken up. Thus, although some species are very effective in taking up DNA from the environment, they remain defined species because integration of foreign DNA is very infrequent and seldom does such integrated DNA confer a selective advantage on the new host. Other factors that are likely to reduce the frequency with which foreign DNA can be maintained in populations are differences in regulatory signals controlling the expression of genes between the parent and the host species, or different levels of gene expression depending on different preferences for codon usage (Ammann *et al.*, 2003).

In crops, the homology of the genomes between related species leads to a wide range of possibilities for the introgression of a transgene, or any other gene after the first hybrid generation. Meiotic abnormalities caused by the distant relationship between parental genomes decrease rates of introgression into new genotypes, thus the production of initial hybrids does not ensure that transgenes will move into weeds or wild relatives. Meiotic abnormalities of hybrid plants may result in higher rates of infertility and decreased rates of seed production. Recombination – an important process in the incorporation of foreign DNA - is reduced by the unstable chromosome configuration of hybrids produced by distant relatives (Chrispeels and Sadava, 2003). When crosses between plants result in a stable incorporation of genes from one pool to another, differently composed gene pool, the process is called introgression or introgressive hybridization. Introgression is often difficult to prove with certainty because shared traits may also be the result of common ancestors or convergent evolution. The most powerful way to detect introgression is by tracking linked molecular markers.

CONSEQUENCES OF TRANSGENE TRANSFER
Possible consequences include the evolution of weeds with increased invasiveness and persistence, extinction of wild relatives, and transgene establishment in wild species with subsequent ecological impacts and disturbances.

The **consequences of the transfer of transgenes** to weeds or wild relatives depend on the nature of the novel gene and the biology and ecology of the recipient plant. Gene flow from GM plants to wild relatives has two potentially harmful consequences: the evolution of weeds with increased invasiveness and persistence, and the likelihood of extinction of wild relatives. The transfer of herbicide tolerance genes,

for example, is unlikely to confer any competitive advantage to hybrids outside agricultural areas. On the other hand, the transfer of traits such as resistance to particular pests and diseases or stress tolerance could potentially give selective advantages to a given plant (increased fitness). Transgenes related to agricultural practices (herbicide tolerance) will likely not affect non-agricultural environments. In the case of herbicide tolerance, wild weed species may become superweeds. Transgenes that provide fitness-enhancing characteristics under natural conditions have the potential to disrupt the balance of established ecosystems. For insect resistance, wild species may become unpalatable and this would affect non-target invertebrates if their host plants take up the resistance gene. Crops that are being engineered for attributes such as modified starch content, reduced pod shatter, virus resistance, etc. may affect wild relatives and cause ecological imbalances.

6.4 EVALUATION OF GENE TRANSFER

EVALUATION OF GENE TRANSFER
To assess the potential for gene transfer, studies should focus on the distinctive characteristics of pollen production, the pollen dispersal and the potential for outcrossing for each GM crop in each location.

Studies in risk evaluation for gene flow must consider primarily, for each crop in each location, the distinctive characteristics of pollen production, as well as the pollen dispersal and the potential for outcrossing. There are three main types of crops:

- Crops with no sexually compatible wild relatives;
- Crops with wild relatives but with poor compatibility, although spontaneous hybridization could still occur, e.g. oilseed rape and wild turnip;
- Crops with fully compatible wild relatives, e.g. sugar beet, which hybridizes readily with wild sea beet.

The possible implications of hybridization and introgression between crops and wild plant species are so far unclear because it is difficult to predict how the transgenes will be expressed in a related wild species. The fitness of wild plant species containing introgressed genes from a GM crop will depend on many factors involving both the genes introgressed and the recipient ecosystems. While it

BOX 6.3

MEASURES TO LIMIT GENE FLOW IN PLANTS: BIOLOGICAL AND PHYSICAL BARRIERS

MEASURES TO LIMIT GENE FLOW IN PLANTS
This includes a variety of biological and physical barriers; details are provided in Module D.

- **Separating distance:** Especially important when the purity of a crop is paramount, e.g. organic crops grown in the vicinity of transgenic crops.
- **Barrier crops** planted around the transgenic crop and intended to capture any pollen drift. Thus, border rows act as buffers to pollen dispersal.
- **Crop isolation zones** between the GM crop and non-GM crop neighbour, creating a geographic barrier to ensure purity of non-GM crops.
- Manipulation of **flowering time** or blocking flowering.
- Prevent access of **pollinators** to the flowers of the transgenic plants.
  - **Bag flowering structures** to prevent pollen spread by insect vectors, wind, or mechanical transfer, or cover female flowers after pollination to prevent loss or dissemination of GM seed.
  - If seed production is not required, **remove flower heads** before pollen and seed production.
- **Harvest** plant material of experimental interest before sexual maturity;
- **Locate test plots** surrounded by roads or buildings to ensure isolation.
- **Cleistogamy** incorporated into the crop so that flowers remain closed during pollination (as happens, for example, in wheat and soybeans).
- **Hybrid barriers**: Pre-fertilization interspecific incompatibility at the stigma surface or within the style, or post-fertilization barriers that cause seed abortion.
- Genetically engineered **male sterility** so that plant produces infertile anthers.
- **Seed sterility** so that the GM plant produces seed that cannot germinate.
- **Apomixes**, the production of seed without fertilization.

For a detailed discussion of GMO containment measures, please refer to Module D.

is important to determine frequencies of hybridization between crops and wild relatives, it is more important to determine whether genes will be introgressed into wild populations and establish at levels which will have a significant ecological impact (Eastham and Sweet, 2002). The information needed to assess potential environmental risks associated with outcrossing from transgenic plants include: biogeographical information on the species involved, reproductive biology of the plant and distribution of sexually compatible relatives, and the impact of the introduced trait, if introgressed into other plant species. Currently there are several useful tools available for evaluation, such as the Geographical Information System (GIS), modelling, and data related to geographical origin, and region of cultivation. Considerable information is already available on the biology of all major crops, making it relatively straightforward to characterize the likelihood of gene flow for any given crop using published literature and simple field surveys. Overall, the potential impacts of gene flow from GM crops are assessed in two steps:

BOX 6.4

KEY QUESTIONS ABOUT GM CROP - WILD SPECIES GENE FLOW

- Is the crop cultivated in vicinity to its wild relative and do they flower at the same time? How far can pollen from the crop travel?
- How easily can crop alleles introgress into wild/weedy populations? Do some crop alleles persist indefinitely?
- What is the baseline fitness of crop-wild hybrids compared with the wild relative?
  Are there strong interactions?
  Are later generations more fit than early ones?
- Are transgenic traits associated with fitness benefits and/or fitness costs? Could fitness-enhancing traits exacerbate weed problems (spread of herbicide resistance) or harm non-target organisms (pollinators)?
- Considerations related with viability and fertility of the hybrid progeny:
  Are the seeds produced viable?
  Will the plants be fertile and produce viable seeds?

(1) the potential for gene flow to occur (likelihood) between the GM crop and any wild relatives is estimated (the exposure component), and (2) the potential environmental impact of gene flow (the hazard component), if it were to occur, is assessed. Gene flow will be higher from crops possessing characteristics that include high pollen production, an ability to disperse pollen over long distances, pollen production over a long period of time, and/or abundant, outcrossing wild relatives.

The development of effective strategies for the safe use of GM crops will depend on adequate biological and ecological characterization of the systems of interest that can only be achieved through a combination of appropriate field tests conducted in relevant environments and development of appropriate models and monitoring methods (Ammann *et al.*, 1999). The Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants of the National Research Council (NRC-CEI, 2002) found that ..."the transgenic process presents no new categories of risk compared to conventional methods of crop improvement but that specific traits introduced by both approaches can pose unique risks".

CHAPTER 7

ECOLOGY OF GM CROPS – ENVIRONMENTAL EFFECTS

Prior to the advent of genetic engineering, plant breeding was not subjected to a great deal of regulation. Seed certification standards ensured the purity and quality of seeds, but little attention was paid to the possible food safety or environmental impacts of new plant varieties derived from conventional breeding. Conventional plant breeding differs considerably from natural selection. Artificial selection and conventional plant breeding break down the resilience in agro-ecosystems, thereby creating gene combinations that would rarely survive in nature. Conventional breeding has been responsible for a few cases of negative effects on human health. The concerns associated with genetically transformed crops are equally applicable to conventional crops. Most of the world's major food crops are not native to their major production zones; rather, they originated in a few distinct "centres of origin" and were transferred to new production areas through migration and trade. Highly domesticated plants are grown all over the world and migration outside cultivated areas has only rarely caused a serious problem (FAO, 2004; NRC-CEI, 2002). While there are risks associated with the introduction of any novel organisms into a habitat, the ecology of genetically engineered organisms is exactly the same as the ecology of any other living thing (FAO, 2004). The ecological rules are precisely the same, no matter how the genotype is put together. Nevertheless, the ecological risks associated

with a GMO, associated either with the GMO itself or a possible introgression of transgenes from the GMO to related species, need to be assessed and evaluated on a case-by-case basis.

ECOLOGICAL RISKS OF GM CROPS
These include persistence, invasion, gene flow, reduction of biodiversity, development of pests resistant to GM crops, development of superweeds and effects in non-target organisms, amongst others.

Ecological risks of GM crops that need to be considered are:

- persistence: the transgenic plants become serious arable weeds;
- invasion: the transgenic crops become invasive of natural habitats;
- gene flow: transfer of introduced genes via pollen (or some other process) to other plant species (so that these then become persistent or invasive);
- reduction of *in situ* biodiversity;
- development of pests resistant to GM crops;
- effects on non-target organisms.

The risks are currently not perceived as being high; transfer of genes resulting from conventional crop breeding into non-crop plants has not created conspicuous problems, nor have traditional crop plants themselves become invasive of natural habitats (FAO, 2004). To date, none of the potential risks has been manifest to any significant extent. Of course, this does not imply that these risks do not exist and that thorough investigations and safety measures need not be taken before releasing a GMO into the environment.

The foremost environmental issue is the presence of sexually cross-compatible relatives, whether domesticated or wild. The wild types may be directly related to a crop as progenitors, or they may be indirectly related as neighbouring taxa. Domesticated relatives are local, farmer selected cultivars, also called landraces. Both wild and domesticated relatives fulfill important roles as reflections of socio-cultural identities, production capital of farmers, and repositories of genetic diversity for plant breeders and farmers alike. An important feature of these domesticated or wild relatives is that they generally cross readily with introduced cultivars. This feature sets the stage for potentially extensive gene flow in domestication centres

between transgenic cultivars and their relatives. On the other hand, crops have evolved to increase self-pollination, which reduces gene flow among crop varieties.

The concerns related to GMOs can also be classified by **type of impact**:

TYPES OF IMPACT of GMOs include impact on the environment, impact on agricultural practices, and impact due to interactions.

- **Impact on the environment.**
  - Persistence of the transgene (better adaptation, invasiveness) or the products of the transgene (cumulative effects);
  - Susceptibility of non-target organisms;
  - Increased use of agro-chemicals;
  - Unpredictable expression of the transgene or transgene instability.
- **Impact on agriculture and agricultural production.**
  - Development of resistance or tolerance in target and non-target organisms;
  - Development of weeds and superweeds;
  - Reduction in nutritive value;
  - Reduction in number of varieties (increase in susceptibility to pest and diseases) and loss of biodiversity (for preference of GM crops over conventional crops);
  - Increased costs of agricultural production;
  - Lack of capacity for risk evaluation and management;
  - Ethical aspects, dependence on seeds, labelling (rights to information).
- **Impact due to interactions**
  - Genetic contamination through pollen and seed dispersal and horizontal transfer of genes;
  - Transfer of the transgene to micro-organisms or generation of new viruses;
  - Interaction among different GMOs.

To assess and evaluate these potential impacts and the likelihood with which they will occur, a detailed ecological risk assessment needs to be conducted for each newly developed GMO that is considered for commercial release.

BOX 7.1

AIMS OF ECOLOGICAL RISK ASSESSMENT

ECOLOGICAL RISK ASSESSMENT
To investigate and assess the ecological risks associated with the release of a GMO; for details please refer to Module C.

- to determine the potential for persistence and spread of transgenic crops in a variety of habitats;
- to determine the range of plant species that can cross-pollinate with transgenic crops;
- to investigate the ecological performance of hybrid plants produced by such pollination;
- to develop protocols that would allow crop breeders to carry out their own ecological risk assessments on new transgenic plants in the future.

The risk assessment studies need to consider the fate of the genetically engineered plants (and their pollen) and the effects of the introduction on the environment (i.e. on subsequent crops in the same fields, on adjacent crops, and in nearby natural habitats), considering:

- problems concerned with the persistence of the vegetative plant and its propagules in different kinds of environments;
- problems related to the spread of the plant by vegetative growth and by seed in both arable fields and natural habitats;
- problems involving the risks of lateral spread of the engineered genes, either by pollination of different plant species or by other means.

Certain principles guide the risk assessment. First, the risk assessment must be specific to the crop and trait involved and the region where introduction is going to occur on a case-by-case basis. Because the environmental impact of the product will depend upon local conditions and practices, the ecological risk assessment must consider the nature of local agro-ecosystems and farming practices within these systems. Differences in cropping practices and native flora and fauna must

be taken into consideration when identifying potential hazards and prioritizing research needs. Second, it is not possible to demonstrate absolute safety for any technology or activity, as all technologies and activities carry some risk. Instead, relative safety compared with alternative technologies is what must be assessed. A regulator must consider whether the product involves greater risks than comparable technologies. Alternatively, the regulator may compare the net benefit (benefit-risk balance) for the product. Note that this risk-benefit balance will reflect local views on the importance of risk and uncertainty, and thus regulators in different regions may make different decisions based on the same data. The assessment then should consider the relative risks and benefits of the new product relative to current practices, and should include the potentially important ecological impacts of these technologies. For an insect-control product like Bt cotton, current practices typically involve the use of conventional insecticides. For herbicide-tolerant crops, there would be other herbicide regimes. These comparisons must be carried out based on local conditions.

7.1 CONCERNS AND POTENTIAL RISKS OF GMOs TO THE ENVIRONMENT

7.1.1 Persistence of the crop/transgene

In the evaluation of possible impacts of a transgenic plant, one of the fundamental issues is to establish whether the introduced genes (traits) can result in the crop becoming more persistent (weedy) in an agro-ecosystem or more invasive in natural habitats. It is known that the characteristics of a weed are the sum of many different traits and that the addition of a single gene is unlikely to turn a plant into a weed. Special attention should be paid, however, to those crops that already have some weed traits or those in which addition of a gene might increase competitiveness in agro-ecosystems or their invasiveness in natural ecosystems. For example, crops that have a short history of domestication are closest to this situation as they still have many "wild" genes and traits, conferring

PERSISTANCE
Potential of a GM crop to grow outside of an agricultural setting and thus become a potential weed.

competitiveness, that are usually eliminated during selection processes to improve a crop. Those GM crops used to date do not show evidence of having increased in persistence or invasiveness. It is important to consider whether a crop is sown in its centre of origin or domestication, and the type of environment that it is introduced into. For this reason risk must be studied and evaluated on a case-by-case and step-by-step basis.

7.1.2 Gene flow and gene dispersal from transgenic crops

Gene flow and gene dispersal are two separate phenomena and their potential consequences are different. Gene flow refers to exchange of genes (transgenes) among species, usually mediated in the case of plants by hybridization, whereas **gene dispersal** refers solely to movement of pollen. Concerns for gene flow are that there will be genetic pollution of species through creation of "unnatural" hybrids and that new superweed species could be created that would have direct consequences for the environment and agriculture. If gene dispersal has any effect, it is likely to be short-term, but effects of gene flow could be long-lasting and persistent. Introduced genes could potentially spread in adjacent populations creating new phenotypes. Investigating and evaluating this process requires insight into ecological impacts of such events, including studies of population sizes, dynamics, and spread and development to quantify and predict possible scenarios.

GENE DISPERSAL
The movement of (transgenic) pollen.

An additional factor in the need to restrict inadvertent gene flow is the possibility of generating feral populations of the crop. Many crops do not survive long periods off-farm, but under semi-natural conditions seed may remain dormant but viable for long periods and feral populations of the crop might eventually establish. This represents a potential problem especially among members of the cabbage family, where species such as rape have become serious weeds. If weedy species contain herbicide resistance genes, for example, they could pose a particularly serious management problem. If these genes were passed

among different species within a genus, or among related genera, hybrid weeds could be created. Similar concerns as those that have been voiced for herbicide tolerance genes will likely be heard should **genetic use restriction technology (GURT)** genes be deployed in crop plants. The major fear, again, in this case is that these genes could be transferred to non-genetically modified crops of the same or related species. The spread of resistance or tolerance genes to pests and diseases has to be considered in a double sense. There are possibilities for those genes to render related weed species more resistant, but depending on the case, they could represent possibilities for better survival of wild species. In general terms, it is likely that they represent an environmental impact only when a new transgene confers enhanced fitness to a crop or its wild relatives with which it is sexually compatible.

GENETIC USE RESTRICTION TECHNOLOGY (GURT)
A proposed technology applying transgenesis to genetically compromise the fertility or the performance of saved seed of a cultivar or of second generation animals. The intention is to protect the market for the seed producer or to prevent undesired escape of genes. Two types of GURTs have been patented: variety-level GURT (V-GURT), which produces sterile progeny, and trait-specific GURT (T-GURT), in which only the added value transgenic trait is genetically protected.

In general, assessing the impacts of introducing new technologies into centres of diversity requires a special degree of care for several reasons. There is widespread consensus among scientists and policy-makers that the biological and genetic diversity of these regions needs to be preserved, and may be vulnerable to ecological disturbances. Centres of diversity, and centres of origin for crop species, represent areas where many potentially-impacted wild species may exist, including wild relatives of crop species that may be recipients of gene flow, as well as many non-target species that could be directly or indirectly impacted by changes in agro-ecosystems (Lemaux, 2008).

7.1.3 Susceptibility of non-target organisms

Toxicity to living organisms refers to inadvertent effects caused by GMOs to benign organisms in the environment. This can be the case if a GM crop carries resistances to pests and diseases. The ideal situation in GM development is to identify a resistance gene to a pest or disease and introduce it so that it is expressed solely in the tissues where needed. Only then is it likely to have an effect only on the

SUSCEPTIBILITY OF NON-TARGET ORGANISMS
Constitutes a major concern regarding the introduction of pest or disease-resistant GM crops. Any effects of such a crop on non-target, possibly beneficial organisms must be carefully assessed and evaluated.

target organism and not on non-target organisms; although non-target organisms feeding on the tissue might still be affected. To achieve this, however, is not easy. There are current advances in this area and there are commercial cultivars that show tissue-specific gene expression. For example, there are numerous maize lines that express toxins from *Bacillus thuringiensis* (Bt) specifically to combat insect infestation, others with increased expression of genes for lysine production in the grain, canola that expresses genes leading to male sterility in the pollen, maize with higher oil content in the grain and others with a changed fatty acid profile and starch structure.

The most studied examples of genetically manipulated resistance in crops are those employing the Bt delta endotoxins. The Bt soil bacterium is abundant under natural conditions and produces a toxin that is lethal to certain insect pests with specific characteristics. One of the most discussed experiments involved Bt toxins and the monarch butterfly (a non-target organism) in the United States. The results of a laboratory study published in 1999 suggested that Bt maize represented a danger to the monarch larvae that consumed *Asclepias* spp. that were covered with transgenic maize pollen. The study did not determine the ecological consequences of the results and the tests were done under laboratory conditions that did not equate with natural conditions (Losey *et al.*, 1999). The publication based on the results of the experiments generated global interest and stimulated the set-up of a cooperative research programme in the same year. The research centred on the effects of the supposedly toxic transgenic maize pollen on monarch larvae feeding on pollen-dusted leaves of their food plant. The authors concluded that although the Bt pollen could be toxic at certain high concentrations, under field conditions there was little risk to the monarch larvae as such high concentrations of pollen would be unlikely to occur in nature (Stanley-Horn *et al.*, 2001). The ideal resistance mechanism for pest control would be one with no unwanted adverse effects on other organisms or the ecosystem.

A second example: ladybirds are generally considered to be beneficial organisms; many eat aphids that are capable of damaging crops through direct feeding and vectoring viruses. Recent studies on the effect of the Bt toxin Cry1Ab from transgenic maize on the biology of the ladybird *Stethorus punctillium* indicated that the toxin had no effect on its fitness. It was shown that the ladybird lacked the midgut receptors for the active toxin to bind to (Alvarez-Alfageme *et al.*, 2008). This research indicates that there is a long way to go before the effects of transgenic crops on non-target organisms are well understood.

A further area that must not be overlooked is the effect of dead transgenic plant material on soil organisms (detritivores) feeding on it. The expression of a transgene in plant tissue might have effects on the small animals and micro-organisms decomposing it, with possible secondary effects for the entire soil community. Therefore, the effect of a GMO on the soil community needs to be carefully assessed and evaluated.

7.1.4 Unforeseen gene expression and instability of transgenes

UNFORESEEN GENE EXPRESSION AND INSTABILITY OF TRANSGENES
After producing a GMO, it needs to be verified that the transgene is expressed in the desired temporal and spatial manner, and that it is stably integrated in the genome and passed on from one generation to the next.

This potential risk relates to concern over wide crosses in conventional crop breeding. In conventional breeding techniques it is not possible to determine *a priori* which genes will be introduced by a cross. This implies a long process of targeted selection after crossing to remove unwanted genes and traits. With GM crops, however, it is known with almost certainty which genes are introduced and it is the subsequent laboratory work that determines which will be expressed and will be stable. In general terms, given that there is ample knowledge of the genes and DNA sequences used in genetic transformation, the number of genes introduced into a GM plant is smaller than in a conventional cross. Technical developments mean that a transgene insertion can be specifically located and its

expression quite accurately controlled. What distinguishes this technology from the conventional technology is the improved precision in introduction of a small number of well-known genes to make for a much better controlled process. To date there appears to be no evidence of phenomena like uncontrolled gene expression or transgene instability in the GM crops studied and evaluated. Nevertheless, each GM crop needs to be carefully evaluated to verify the expression pattern of the introduced genes and the stability of the transgene.

7.1.5 Weeds

WEEDS
Concerning weed management, biotechnology so far has been mainly applied to create herbicide-resistant crops. Future goals include improved resistance to parasitic weeds.

Weeds fall into two major classes, parasitic and non-parasitic. Weed control is a major component of crop management programmes. Biotechnology has been less successfully applied to weed management than to management of other biotic stresses. For non-parasitic weeds, biotechnology has been applied to develop herbicide resistance, an indirect control strategy where the crop is the target of the transgene and not the weed. Species of two parasitic weed genera, *Striga* and *Orobanche*, represent important weeds of the tropics and Mediterranean areas. They are currently managed through various strategies including manual weeding, crop rotations, chemical control and biocontrol. Biotechnology has the potential to transform crops to allow herbicide application for weed control and to alter gene action controlling the stimuli that trigger germination and development of parasitic weed seed. More knowledge of the host-parasite relationship at the molecular level will allow more environmentally sound management methods to be developed.

Parasitic weeds represent a very specific management challenge. Each plant of *Striga hermontheca*, a major problem of cereal crops in the tropics, is able to release 100 000 seeds into the soil, each of which can remain viable for up to fifteen years. There is variation in resistance of some crops, including sorghum, which appears to be under genetic control. This can be selected for using traditional plant

breeding methods, but can probably be enhanced in the future using methods from molecular biology such as MAS. For crops, including maize, there is no naturally occurring host-plant resistance and the only possibilities of obtaining any, though this has not been done yet, would be to induce it or transfer non-host resistance, which occurs in many grass species. Unfortunately, very little is known about the mechanisms of non-host resistance.

Transforming crops to tolerate contact herbicides would not be effective in managing parasitic weeds as they have already done their damage before they appear above the soil surface. Transforming the crop for application of systemic herbicides, as has been done for non-parasitic weed management, is unlikely to be effective as the crop breaks down the herbicide into harmless chemicals that do not consequently reach the parasite, which is intimately linked with the crop via its roots. Enzymes in the crop that are associated with herbicide uptake could be modified to prevent herbicide binding and promote build-up of the herbicide in the parasite. Glyphosate resistance works in this way, which is termed target-site resistance. This represents the most feasible form of control and has been effective in controlling *Striga* and *Orobanche* infestations in various crops sprayed with several herbicide formulations. Seed dressings that rely on this mechanism can also be used.

One issue constantly being raised is that of the development of a **superweed** which, created through flow of herbicide tolerance transgenes, would become impossible to control using standard herbicides. To date such a weed has not developed, but serious weed problems have arisen through deliberate introduction of new ornamental plants and inadvertent introduction of exotics. Some of these have literally become some of the world's worst weeds and yet have been relatively unnoticed by environmental lobby groups. They represent introductions of entire new genomes, and are not merely the result of (trans)gene flow. Perhaps, in the future, there will generally be a better understanding of the relative level of risk posed by the flow of ethically contentious genes.

SUPERWEED
The development of a herbicide-resistant weed through flow of herbicide tolerance genes from a GM crop to a weedy relative.

BOX 7.2

QUESTIONS ADDRESSED BY FIELD TESTING

FIELD TESTING
Realistic, small-scale field tests are necessary to evaluate potential risks as well as benefits for commercial-scale uses of GMOs.

Some questions related to the release of genetically engineered organisms can be answered only with practical experience. Realistic, small-scale field tests are the way to evaluate potential risks from commercial-scale uses of genetically engineered organisms. However, these short-term studies are only appropriate to risk assessments on annual crop plants.

At the end of a three-year study of the population biology of transgenic and non-transgenic annual crop plants, one should be in a position to:

- Provide data on persistence and invasion in natural and arable habitats.
- Show how (and if) genetic engineering alters these parameters.
- Describe pollen spread by insect vectors and by other means.
- Show how (and if) genetic engineering alters the production, spread, or compatibility of pollen.
- Catalogue the wild plants that share insect pollinators with the crop.
- Provide quantitative data on successful cross-pollination between the crop and its wild relatives.
- Provide data on the persistence and invasiveness of any transgenic hybrid plants produced by crossing experiments.

7.2 POTENTIAL BENEFITS OF GMOs

The use of industrial agrochemicals has a substantial bearing on the sustainability of agro-ecological systems. Pesticides have not only had direct negative impacts on the quality of the environment, but have also adversely affected biodiversity through removing beneficial and inoffensive organisms. Interestingly, glyphosate, which several crops have been transformed to tolerate, is much less toxic than

some of the herbicides (e.g. atrazine) it replaced. There is concern that GMO use in the field of herbicide resistance will result in increased use of herbicides. Evidence suggests that this has not been the case, but that herbicide use has been reduced at the commercial level. Reduction in pesticides can be obtained by identifying, developing and deploying durable host-plant resistance to pests and diseases. Insect pests (9 000 species), plant pathogens (50 000 species) and weeds (8 000 species) account for the greatest crop losses, and their control requires the greatest use of agro-chemical crop protection. The advantages of host-plant resistance are numerous and include: it is relatively inexpensive for the farmer in comparison with chemical control; it is always present; it has no effect on organisms other than the target ones; it can be extremely durable; it can employ a diversity of resistance genes; it does not interfere negatively with other forms of control; and it has no negative effects on yield. There are also many possibilities to improve crop production through breeding for adaptation to a range of abiotic stresses, including drought, salt and heat, and more efficient use of nitrogen and water.

Biotechnology applications to date have focused on engineering traits such as herbicide resistance for some of the major commodity crops, but there is considerable potential for expanding the methods to include a broader range of crops and genetically more complex traits. Many disease resistances are governed by few genes and represent relatively easy targets for the molecular breeder. Resistance to some diseases is controlled by many genes, each to little effect. Using modern methods, including quantitative trait loci (QTL) analysis, important areas of the genome in resistant lines can be identified, located and ultimately cloned for inclusion into susceptible, but otherwise adapted, germplasm.

Table 7.1 | Potential direct and indirect effects of GM crops on the environment[1]

ENVIRONMENTAL EFFECTS OF GMOs				
Direct Effects				
Invasiveness of GM crops	**Gene flow**	**Environmental fate of transgenic products**	**Effects on non-target organisms**	**Effects on target organisms**
Survival outside the cultivated area	Pollen transfer to wild relatives and hybrid formation	Persistence, degradation and spread of transgenic pollen	Direct or indirect uptake of transgenic products through feeding	Resistance development in target organisms
Reproduction outside the cultivated area	Survival and reproduction of hybrids	Accumulation of transgenic products in soil	Effects on non-target organisms	Effects on population dynamics
Transgenic population (hybrids/crops) with enhanced fitness compared to wild population		Eluviation of transgenic products from soil	Effects on population dynamics	
Spread and persistence of transgenic plants (hybrids/crops) outside the cultivated area		Immission of transgenic products into water	Effects on ecosystems	
Outside the cultivated area (substitution of natural population)	In the cultivated area (super weeds)			
Environmental damage	**Economic damage**	**Environmental damage**	**Environmental damage**	**Economic damage**

1 Adapted from Sanvido O., M. Stark, J. Romeis and F. Bigler (2006) Ecological impact of genetically modified crops – experiences from ten years of experimental field research and commercial cultivation. ART Schiftenreihe 1, Agroscope Reckenholtz-Tänikon Research Station ART, Zurich, Switzerland.

ENVIRONMENTAL EFFECTS OF GMOs				
Indirect Effects				
Resistance development		**Effects on agricultural methods and cropping systems**		
Resistance development on target pest/ disease	Selection of herbicide tolerant wild plants	Changes in cultivation practice/tillage	Changes in cropping intervals/ cultivation area	Excess of agronomic competitiveness of GMOs
Loss of effectiveness of transgenic products	Reduced effectiveness of specific herbicide	Changes in spectrum of pests, diseases and beneficial organisms	Changes in physical, chemical and biological soil characteristics	Substitution of cultivations which have no GMO available
Need to change pest/disease control strategies			Decrease in soil quality	Substitution of traditional varieties
		Effects on biodiversity		
Economic damage		**Environmental damage**		

REFERENCES

Alvarez-Alfageme F., Ferry, N., Castañera, P., Ortego, F., & Gatehouse, A.M. 2008. Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). *Transgenic Res.* 17(5): 943-54.

Ammann, K. 2003. *Biodiversity and agricultural biotechnology: a review of the impact of agricultural biotechnology on biodiversity.* Botanischer Garten Bern, Switzerland. 54 p.

ASAP (Agroecology/Sustainable Agriculture Program). 2004. Principles of sustainable agriculture (available at: http://www.aces.uiuc.edu/~asap/index.html).

Begon, M., Townsend, C.A. & Harper, J.L. 2006. *Ecology: from Individuals to ecosystems*. 4th Edition. Wiley-Blackwell.

CBD (Secretariat of the Convention on Biological Diversity). *Biodiversity and Agriculture: Safeguarding Biodiversity and Securing Food for the World.* Montreal, 2008.

CBD (Secretariat of the Convention on Biological Diversity). Texts and documents (available at: www.cbd.int).

Chrispeels, M. J. & Sadava, D.E. 2003. *Plants, genes and crop biotechnology*. Second Edition. American Society of Plant Biologists and the ASPB Education Foundation. Salisbury, Massachusetts, USA, Jones and Bartlett Publishers.

Eastham, K. & Sweet, J. 2002. *Genetically Modified Organisms (GMOs): The significance of gene flow through pollen transfer.* European Environment Agency EEA´s Corner Series Environmental Issue Report Nº 28. 75 p.

FAO. 2001. *Genetically modified organisms, consumers, food safety and the environment* (available at: www.fao.org).

FAO. 2004. *The state of food and agriculture 2003-2004 (SoFA)*. Agricultural biotechnology: meeting the needs of the poor?

FAO, 2007. *Marker-assisted selection. Current status and future perspectives in crops, livestock, forestry and fish.*

Fraleigh, B. 2006. Global overview of crop genetic resources, as part of FAO publication *The role of biotechnology in exploring and protecting agricultural genetic resources*.

Henry, R. J. 2000. *Practical applications of plant molecular biology*. London, Chapman & Hall. 258 p.

Kameswara Rao, C. & Shantharam, S. 2004. *Can GM crops be introduced into crop centres of origin and diversity?* Special to AgBioView, 13 April 2004.

Lemaux, P.G. 2008. Genetically engineered plants and foods: a scientist's analysis of the issues (Part I). *Annu. Rev. Plant. Biol.* 59: 771–812.

Losey, J.E., Ravor, L.S, & Carter M.E. 1999. Transgenic pollen harms monarch larvae. *Nature* 399(6733): 214.

NRC-CEI (National Research Council Committee on Environmental Impacts). 2002. *Environmental effects of transgenic plants*. Washington, DC, National Academy Press. 320 p.

NRCS (Natural Resources Conservation Service – USDA). 2004. *Agricultural ecosystems and agricultural ecology* (available at: www.nrcs.usda.gov).

Purves, W.K., Sadava, D., Orians, G.H. & Heller, H.G. 2004. *Life: the science of biology*. Sunderland, Massachusetts, USA, Sinauer Associates, Inc. 7th Edition. 1121 p.

Rao, N.K. 2004. Plant genetic resources: advancing conservation and use through biotechnology. *African Journal of Biotechnology* 3(2): 136-145.

Ricklefs, R.E. & Miller, G.L. 1999. *Ecology*. New York, USA, W. H. Freeman & Co. 4th Edition. 822 p.

Stanley-Horn, D.E., Dively, G.P., Hellmich, R.L., Mattila, H.R., Sears, M.K., Rose, R., Jesse, L.C., Losey, J.E., Obrycki, J.J., & Lewis, L. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proc. Natl. Acad. Sci. U S A* 98(21): 11931-6.

The National Academies. USA. 2007. *New horizons in plant sciences for human health and the environment* (available at: http://www.nationalacademies.org/plant_genome).

WCMC (World Conservation Monitoring Centre). 2002. Biodiversity: an overview. (available at: www.wcmc.org.uk/infoserv/biogen/biogen.html).

USEFUL READINGS

ACRE (Advisory Committee on Releases to the Environment UK). 2007. *Managing the footprint of agriculture: towards a comparative assessment of risks and benefits for novel agricultural systems*. Report of the ACRE Sub-Group on Wider Issues raised by the Farm-Scale Evaluations of Herbicide Tolerant GM Crops (available at www.defra.gov.uk/environment/acre).

AGBIOS (Agriculture & Biotechnology Strategies, Inc.). 2002. Essential Biosafety™. A comprehensive source of scientific and regulatory information. CD-ROM2nd Edition.

Agrifood Awareness. 2004. New co-existence study on GM and non-Gm crops in the EU (available at: http://www.pgeconomics.co.uk/pdf/Co-existencestudyEU_PG_Economicsmay2004.pdf).

Ammann K., Jacot, Y., Simonsen, V. & Kjellsson, G. (Eds.). 1999. *Methods for risk assessment of transgenic plants*. III. Ecological risks and prospects of transgenic plants, where do we go from here? A dialogue between biotech industry and science. Basel, Switzerland, Birkhaüser-Verlag. 260 p.

Ammann, K. 2007. Reconciling Traditional Knowledge with Modern Agriculture: A Guide for Building Bridges. In *Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices* (eds. A Krattiger, RT Mahoney, L Nelsen, *et al.*). Oxford, U.K., MIHR, and Davis, USA, PIPRA (available at www.ipHandbook.org).

Ammann, K., Jacot, Y. & Braun, R. (Eds.) 2003. *Methods for risk assessment of transgenic plants*. IV. Biodiversity and biotechnology. Basel, Switzerland, Birkhaüser-Verlag. 177 p.

Benbrook, C.M. 2002. A perspective on actual versus potential environmental benefits of agricultural biotechnology. In: *Environmental Savior or Saboteur? Debating the Impacts of Genetic Engineering*. 4 February, San Francisco, California (available at: http://www.biotech-info.net).

Bradford, K.J., Van Deynze, A., Gutterson, N., Parrot, W. & Strauss, S.H. 2005. Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology* 23: 439- 444.

Brookes, G. & Barfoot, P. 2006. Global impact of biotech crops: socio-economic and environmental effects in the first ten years of commercial use. *AgBioForum* 9(3): 139-151.

Brookes, G. & Barfoot, P. 2008. *GM crops: global socio-economic and environmental impacts 1996-2006*. UK, PG Economics Ltd.

CIAT-UN-BIODIVERSITY-REDCAPA-CTA. 2007. Multi-Institutional Distance Learning Course on the Ex-situ Conservation of Plant Genetic Resources (available at: www.ciat.org).

Cohen, J.I. 2005. Poorer nations turn to publicly developed GM crops. *Nature Biotechnology* 23: 27-33.

Conner, A.J., Glare, T.R. & Nap, J.P. 2003. The release of genetically modified crops into the environment, Part II. Overview of ecological risk assessment. *The Plant Journal* 33: 19-46.

Dale, P.J., Clarke, B. & Fontes, E.M.G. 2002. Potential for the environmental impact of transgenic crops. *Nature Biotechnology* 20: 567-574.

Daniell, H. 2002. Molecular strategies for gene containment in transgenic crops. *Nature Biotechnology* 20: 581-586.

DEFRA (Department for Environment, Food & Rural Affairs UK). 2002a. *Guidance on best practice in the design of Genetically Modified Crops* (available at: www.defra.gov.uk/environment/acre/bestprac/consult/guidance/).

DEFRA (Department for Environment, Food & Rural Affairs UK). 2002b. *Guidance on the assessment of risk to wider bodiversity from proposed cultivation of GM crops* (available at: www.defra.gov.uk/environment/consult/biodiversity/risk/index.htm)

DEFRA (Department for Environment, Food & Rural Affairs UK). 2002c. *Farm-scale evaluations of genetically modified crops. Answers to some frequently asked questions* (available at: www.defra.gov.uk/environment/fse/raq/index.htm).

Edmeades, S. & Smale, M. 2006. A trait-based model of the potential demand for a genetically engineered food crop in a developing economy. *Agricultural Economics* 35(3): 351–361.

Halpin, C. 2005. Gene stacking in transgenic plants – the challenge for 21st century plant biotechnology *Plant Biotechnology Journal 3*: 141–155.

Hota, M. 2007. *Crop biotech & biosafety*. International Service for the Acquisition of Agri-biotech Applications (ISAAA), New Delhi.

ICSU (International Council for Science - Nuffield Council on Bioethics). 2003. GM Science Review Panel. 298 p.

IFPRI (International Food Policy Research Institute). 2008. *Insecticide use on vegetables in Ghana. Would GM seed benefit farmers?* IFPRI Discussion Paper 00785. Environment and Production Technology Division (available at: www.ifpri.org).

James, C. 2007. *Global status of commercialized biotech/GM crops*. ISAAA Brief No. 37, Ithaca, NY, ISAAA.

Kolady, D.E. & Lesser, W. 2008. Is genetically engineered technology a good alternative to pesticide use? The case of GE eggplant in India. *International Journal of Biotechnology* 10(2/3): 132–147.

Lemaux, P.G. 2001. *Nudging mother nature: the promise and reality of AgBiotech for farmers* (available at: http://ucbiotech.org/resources/biotech/talks/).

Miller, H., Morandini, P. & Ammann, K. 2008. Is biotechnology a victim of anti-science bias in scientific journals? Trends in biotechnology. 122-125 pp (available at http://www.botanischergarten.ch/Peer-Review/Miller-Morandini-Ammann-Peer-Review-2008.pdf).

Nap, J.P., Atanassov, A. & Stiekema, W.J. (Eds.) 2004. *Genomics for biosafety in plant biotechnology*. NATO Science Series. Amsterdam, IOS Press. 247 p.

Pidgeon, J.D., May, M.J. & Dewar, A.M. 2003. GM crop management for environmental benefit. In: 7th ICABR International Conference: Public Goods and Public Policy for Agricultural Biotechnology. Ravello, Italy, 29 June – 3 July.

Potrykus, I. 2004. Experience from the humanitarian Golden Rice project: extreme precautionary regulation prevents use of green biotechnology in public projects. BioVision Alexandria, 3-6 April 2004.

Pullin, A.S. 2002. *Conservation Biology*. Cambridge, U.K., Cambridge University Press. 345 p.

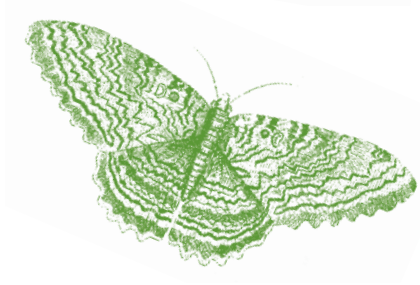
Rull, V. 2008. Speciation timing and neotropical biodiversity: the tertiary–quaternary debate in the light of molecular phylogenetic evidence. *Molecular Ecology* 17: 2722–2729.

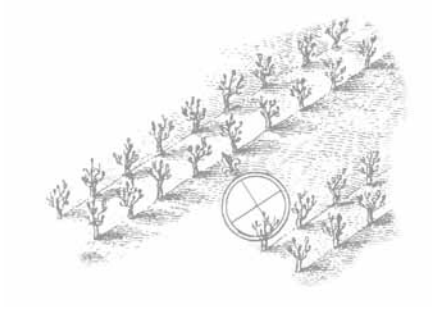
The World Bank. 2007. *World Development Report 2008*. Agriculture for Development (available at: www.worldbank.org

Traynor, P.L., Frederick R. & Koch M. 2002. *Biosafety and risk assessment in agricultural biotechnology (A workbook for technical training)*. The Agricultural Biotechnology Support Project, Institute of International Agriculture, Michigan State University, USA. 142 p.

UN Millennium Project. 2005. *Investing in development: a practical plan to achieve the Millennium Development Goals. Overview.*

Vavilov, N.I. 1997. Five Continents. (Rodin LE,Ed.) International Plant Genetic Resources Institute, Rome, Italy. 198 p.





Design and layout: Pietro Bartoleschi and Arianna Guida (Studio Bartoleschi)
Cover illustrations elaborated from “l’Encyclopédie Diderot et d’Alembert”
Printed in Italy on ecological paper, Forest Stewardship Council (FSC) certified, May 2011

Biosafety Resource Book

MODULE B

ECOLOGICAL ASPECTS

provides the necessary background information on ecology and evolution needed to analyse and understand the consequences of introducing GMOs into the environment.

For additional information please consult **www.fao.org/biotech** or contact **biotech-admin@fao.org**

MODULE C

Biosafety Resource Book

RISK ANALYSIS

FAO
FIAT PANIS

Biosafety Resource Book

MODULE C

RISK ANALYSIS

Alessandra Sensi
Oliver Brandenberg
Kakoli Ghosh
Andrea Sonnino

Food and Agriculture
Organization of
the United Nations
Rome, 2011

LIST OF CONTRIBUTORS

This module has been prepared based on the lectures and contributions of:

Bassam Al-Safadi
Plant Biotechnology Division
Atomic Energy Commission
Damascus, Syria

Nelson Onzere Amugune
Department of Botany
University of Nairobi
Nairobi, Kenya

Esther Argote Pelegrino
Departamento Desarrollo Académico
Instituto Superior de Tecnologías
y Ciencias Aplicadas
Ministerio de Ciencias Tecnologías
y Medio Ambiente
La Habana, Cuba

Rodrigo Artunduaga
Agricultural BioTech
Regulatory Network-ABTR
Vancouver, Canada

Oliver Brandenberg
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
Institute of Medical Virology,
University of Zürich,
Zürich, Switzerland)

Kakoli Ghosh
Plant Protection and Production Division
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

Desiree M. Hautea
Institute of Plant Breeding
University of the Philippines
Los Baños, Philippines

Samuel Kiboi
University of Nairobi
Nairobi, Kenya

Leticia Pastor Chirino
Departamento de Autorizaciones
Centro Nacional de Seguridad Biológica
La Habana, Cuba

Alessandra Sensi
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
EuropeAid Co-operation Office, Unit A.3
Centralised Operations for Europe,
the Mediterranean and the Middle East
Brussels, Belgium)

Sandra Sharry
Universidad Nacional de La Plata
La Plata, Argentina

Andrea Sonnino
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

Fred Tairo
Mikocheni Agricultural Research Institute (MARI)
Dar-es-Salaam, United Republic of Tanzania

CONTENTS

MODULE C

LIST OF ABBREVIATIONS

ABS Access and benefit-sharing
AIA Advanced Informed Agreement
ASEAN Association of Southeast Asian Nations
BCH Biosafety Clearing-House
CBD Convention on Biological Diversity
Codex Codex Alimentarius
COP-MOP Conference of the Parties serving as the meeting of the Parties to the Protocol
CPB Cartagena Protocol on Biosafety
CPM Commission on Phytosanitary Measures
DNA Deoxyribonucleic acid
EC European Commission
EIA Environmental Impact Assessment
EU European Union
FAO Food and Agriculture Organization of the United Nations
FFP Food, or feed or for processing
GATT General Agreement on Tariffs and Trade
GDP Good Development Principles
GMO Genetically modified organism
IP Identity preservation
IPPC International Plant Protection Convention
ISPM International Standard for Phytosanitary Measures
ITPGRFA International Treaty on Plant Genetic Resources for Food and Agriculture
IUCN International Union for Conservation of Nature
LMO Living modified organism
NGO Non-governmental organization
OECD Organisation for Economic Co-operation and Development
OIE Office International des Epizooties
PGRFA Plant Genetic Resources for Food and Agriculture
PRA Pest Risk Analysis
SPM Sanitary and Phytosanitary Measures
SPS Sanitary and Phytosanitary Agreement
TBT Technical Barriers to Trade
TRIPS Agreement on Trade-related Aspects of Intellectual Property Rights
UN United Nations
UNECE United Nations Economic Commission for Europe
UNEP United Nations Environment Programme
UNIDO United Nations Industrial Development Organization
UPOV International Union for the Protection of New Varieties of Plants
USDA United States Department of Agriculture
WHO World Health Organization
WTO World Trade Organization

CHAPTER 1

BIOLOGICAL RISKS: BASIC CONCEPTS AND CLASSIFICATION

1.1 BIOLOGICAL RISKS

The objective of a **biosafety** system is to prevent, manage, minimize or eliminate hazards to human health and security and to protect the environment from biological agents and organisms used in research and trade. The following terminologies associated with biological risks are defined or described:

Biological agents - living organisms, or materials derived from them, which can potentially cause diseases in, or harm to, humans or the environment.

Hazard – a hazard can be described in general terms as "*a situation in which particular circumstances represent a danger*", that is, the potential for an adverse occurrence. One example is a threat to the quality of life of an individual or a group.

BIOSAFETY
To prevent, manage, minimize or eliminate hazards to human health and security and to protect the environment from biological agents and organisms used in research and trade.

BIOLOGICAL AGENTS
Living organisms, or materials derived from them, which can potentially cause diseases in, or harm to, humans or the environment.

HAZARD
A situation in which particular circumstances represent a danger.

BIOLOGICAL HAZARDS
Infectious agents or hazardous biological materials that present a risk, or potential risk, to the health of humans, animals or other organisms.

Biological hazards, or biohazards - *are those infectious agents or hazardous biological materials that present a risk, or potential risk, to the health of humans, animals or other organisms*. The risk can be manifested directly through infection, or indirectly through damage to the environment. Unlike chemical hazards, infectious agents have the ability to reproduce and to give rise to large numbers of infectious organisms/particles, starting from a small amount of initially released material. Biological hazards are numerous and diverse.

An overview of biological hazards is presented in Table 1.1:

Table 1.1 | **Definitions of hazard as applicable to different biosecurity sectors**

Food safety	A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect
Zoonoses	A biological agent that can be transmitted naturally between wild or domestic animals and humans
Animal health	Any pathogenic agent that could produce adverse consequences on animal health
Plant health	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products
Plant health quarantine	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled
"Biosafety" in relation to plants and animals	A living modified organism (LMO) that possesses a novel combination of genetic material obtained through the use of modern biotechnology that is likely to have adverse effects on the conservation and sustainable use of biological diversity, taking into account also risks to human health
"Biosafety" in relation to food	A recombinant DNA organism directly affecting or remaining in a food product that could have an adverse effect on human health
Invasive alien species	An invasive alien species outside its natural past or present distribution whose introduction and/or spread threatens biodiversity

Adapted from: FAO, 2007.

BIOLOGICAL RISK
The risk associated with a biological agent or organism is the probability of the occurrence of a particular adverse event at a specific time and the magnitude of the consequent damage caused.

Biological risks - The risk associated with a hazard can be considered as the potential for a hazard having adverse consequences on human existence and health, property and the environment under specific conditions. The risk is therefore a combination of two factors: the probability and the consequence of an adverse

occurrence. Thus the risk associated with a biological agent or organism is the probability of the occurrence of a particular adverse event at a specific time and the magnitude of the consequent damage caused, depending on various factors such as exposure to the hazard, the frequency of exposure and the severity of any consequent damage done. Many aspects of risk analysis are generic and can be applied to all classes of risk. Risk is a measure of the probability and severity of adverse effects.

It can be expressed as follows: **RISK = likelihood x consequence**

RISK
Likelihood of occurrence x consequence of an incident.

Biological risks can be classified into two broad categories: naturally occurring or human-caused:

- *Naturally occurring biological risks* include:
  (1) the emergence of antibiotic resistant bacterial infections (tuberculosis, pneumonia, flu epidemic);
  (2) naturally emerging pathogens attributed to deforestation (monkeypox, Ebola, Lassa fever);
  (3) spreading of a zoonosis, i.e. infected animal populations conveying the disease to humans via direct contact, vectors or water/foodstuffs;
  (4) toxins arising from certain molds and fungi (deoxynivalenol, aflatoxins, ochratoxin);
  (5) parasitic infection outbreaks in humans;
  (6) invasive alien species (plants, animals and micro-organisms).

- *Human-caused or related biological risks,* which can be further classified into:
  (1) deliberately induced risks such as the use of harmful biological agents through warfare or terrorism; and
  (2) biotechnological risks such as products of traditional cross-breeding and selection, mutation and modern biotechnology.

According to the hazard and the associated risk, hazard-based and risk-based measures can be taken:

WORKING DEFINITIONS FOR HAZARD-BASED AND RISK-BASED CONTROL MEASURES
Hazard based – A control measure that is based on quantified and verifiable information on the level of hazard control that is likely to be achieved but lacking quantitative knowledge of the level of protection that is likely to result.
Risk-based – A control measure that is based on quantitative and verifiable information on the level of protection that is likely to be achieved.

Adapted from: FAO, 2007.

1.2 CLASSIFICATION OF BIOLOGICAL AGENTS

The need to classify biological agents according to their risk arises from the high incidence of diseases contracted by people and because of the possible danger of spreading pathogenic agents in the environment. Furthermore, the growing field of biotechnology and the advances in genetic engineering require detailed analyses of the risks associated with genetically modified organisms. Those in contact with infectious biological agents, genetically modified material or any other potentially harmful biological agent must be made aware of the potential dangers that are associated with and the characteristics of the agents. Education in safe handling of such agents, using appropriate techniques, needs to be made available.

The personnel of research institutions, biomedical firms and veterinary quarantine services are among those that teach, research, produce and control biological materials or foods and feeds. Such materials can potentially represent sources of direct infection, by containing pathogenic micro-organisms. Moreover, the environment could become contaminated if an accidental escape of biological agents were to occur. Therefore, detailed knowledge about the classification of biological agents and material is required to assure appropriate handling and minimize potential risks.

1.3 BIOLOGICAL AGENTS AND RISK GROUPS

One way to **classify biological risks** is based on the risk posed by biological agents to human health and the environment upon accidental or intentional release. Biological agents are typically used in research or biomedical laboratories, and include the full range of micro-organisms: bacteria, viruses, fungi, protozoa and multicellular parasites. Laboratory acquired infections (LAI) have been documented since the beginning of the twentieth century. However, the advent of modern biotechnology raised awareness about the hazards of infectious micro-organisms and the risks they pose to laboratory workers who handle them, and to the community if they escape from the laboratory.

BIOLOGICAL RISK CLASSIFICATION
Based on the risk posed by biological agents to human health and the environment upon accidental or intentional release.

There are three ways that bring workers into contact with materials that may pose a biological risk. These are:

- ***Exposure*** *as a result of working with biological agents* – areas of work include microbiology laboratories, greenhouses and animal houses. Activities include isolation, identification and culture of micro-organisms or cells, including materials used for genetic modification and intentional contact with animals, plants and materials that originate from animals and plants as part of the experimental work.
- *Exposure which does not result from the work itself but is incidental to it, mainly because biological agents are present as contaminants* - areas and activities include farming, refuse collection, sewage treatment, handling human body fluids and excreta, and handling materials that may be contaminated by these materials, such as hypodermic needles or sewage treatment plants.
- *Exposure which is not a result of work* – unintentional contact with animals or animal and plant materials or people, in the workplace or elsewhere.

EXPOSURE
The contact to biological agents that may represent a danger to human health or the environment.

The World Health Organization (WHO, 2004) has recommended an agent **risk group** classification for laboratories, aimed at defining the appropriate containment levels

RISK GROUPS
Four risk groups for biological agents were defined, based on factors such as pathogenicity, mode of transmission, availability of preventive measures and treatment.

required to protect people working with biological agents and ensuring they do not get infected, based on risk criteria/factors described below:

- *Pathogenicity of the agent or its product* - inherent risks of a pathogen are based on factors such as the severity of the disease it causes, its virulence and infectivity. Diseases caused by products of a biological agent include toxicity, allergenicity, and modulation of physiological activity.
- *Mode of transmission and host range of the agent* – these are influenced by existing levels of immunity, density and movement of the host population, presence of appropriate vectors and standards of environmental hygiene.
- *Availability of effective preventive measures* - these may include: prophylaxis by vaccination or antisera; sanitary measures, e.g. food and water hygiene; the control of animal pathogen reservoirs or arthropod vectors; the movement of people or animals; and controlling the importation of infected animals or animal products.
- *Availability of effective treatment* - includes passive immunization and post-exposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of emergence of resistant strains.

Other considerations that may be taken into account in classifying biological agents include:

- *Origin/source* – indigenous (native, local) or exotic (foreign, alien) origin; exotic agents pose higher risks to human health because they may cause more severe infections with no available treatment.
- *Ability of the organism to survive* – dormancy or resting period during unfavourable conditions.
- *Number/concentration of pathogens* – the higher the number and concentration of a pathogen, the greater the likelihood of infection.
- *Nature and route of transmission* – inhalation (dust, aerosol), ingestion (food, drink, saliva), direct contact (cuts, bites, injection).

The *National Institute of Health, USA (*NIH, 2002*)* established a **classification of genetically modified agents** into a particular risk group using the same criteria indicated above. Many countries have adopted the WHO and NIH risk group classifications and criteria.

GMO CLASSIFICATION
Classicication of GMOs into four risk groups, according to the potential danger they represent.

The four resulting WHO and NIH risk groups are presented below in Table 1.2:

Table 1.2 | **Risk group classification of biological agents**

RISK GROUP Classification	NIH Guidelines For Research Involving Recombinant DNA Molecules, 2002	World Health Organization Laboratory Biosafety Manual 3rd Edition 2004
Risk Group I	Agents that are not associated with disease in healthy adult humans	A micro-organism that is unlikely to cause human disease or animal disease. (No or low individual and community risk.)
Risk Group II	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread is limited. (Moderate individual risk; low community risk.)
Risk Group III	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. (High individual risk; low community risk.)
Risk Group IV	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. (High individual and community risk.)

Adapted from: BMBL, 2007.

Using the above criteria of classification, hazard groups can be summarized in the following scheme (Table 1.3):

Table 1.3 | **Hazard group classification**

Hazard Group	Pathogenicity for humans	Hazard to workers	Spread to the community	Effective prophylaxis or treatment
1	Unlikely to cause human disease	Low	Unlikely	Available
2	Can cause human disease	Intermediate	Unlikely	Usually available
3	Can cause severe human disease	Likely/ possibly serious	May spread	Usually available
4	Causes severe human disease	Serious	Likely	Unavailable

The four-risk group classification of biological agents is widely recognized but disagreements exist in allocating agents to a particular risk group. WHO recommends each country draw up its own classification by risk group of the agents encountered in that country based on the above-mentioned criteria and considerations.

1.3.1 Classification of biological agents that affect animals

BIOLOGICAL AGENTS AFFECTING ANIMALS/ PLANTS
As for human pathogens and potentially dangerous biological agents, risk groups for classification of animal/plant pathogens have been defined.

The classification of the WHO is used in the initial stages of establishing laboratory biosafety procedures, but is not strictly applicable to animals. Instead, a working group within the International Veterinary Biosafety Working Group recommended that biological agents that affect animals be classified into four risk groups:

» Low risk animal pathogens: Agents that cause diseases of minor importance for animal health and for which transmission is poor.

- Moderate risk animal pathogens: Agents that cause diseases with a moderate risk of transmission with a certain level of morbidity, but seldom cause mortality.
- High risk animal pathogens: Agents that cause serious, easily transmissible diseases with a high level of morbidity and occasional mortality.
- Very high risk animal pathogens: There is a dual definition for this group. It includes pathogenic agents that cause serious diseases and which can be highly transmissible within the animal population. It also includes micro-organisms that cause serious diseases, are highly transmissible and are associated with high morbidity and mortality.

1.3.2 Classification of biological agents that affect plants

In the case of plants, the classification enables the definition of the risks for the environment resulting from handling of biological agents, facilitating therefore the development of criteria for biosafety procedures in plant facilities. Because some of these agents can affect human health they are included in the classification.

The European Federation of Biotechnology (EFB) developed the first system of classification in 1985, which was then revised in 1992 by the working group on biosafety of the same federation, and they proposed a new system for classification of micro-organisms causing plant diseases (Kuenzi *et al.*, 1987).

The factors affecting development of a disease include:

- inoculum density;
- resistance of the pathogen to environmental conditions (humidity, temperature, cultural practices and chemical application);
- means of dissemination: water, air, soil or vectors;

- presence of susceptible hosts;
- spatial relationship between susceptible hosts and pathogens;
- virulence of the pathogen.

The classification proposed by the working group was:

- Class 1. Micro-organisms that can cause diseases in plants of minor importance. They generally include indigenous species and do not require special biosafety measures to be worked with, except good laboratory practices (GLP).
- Class 2. Micro-organisms that cause important disease outbreaks in crops, ornamental plants and forests. Work with such pathogens is subject to national regulations.
- Class 3. Micro-organisms included on quarantine lists. Importation and handling of these is generally prohibited. Work with them generally requires authorization from national bodies.

For genetically modified organisms (GMOs), the four-risk group classification is employed depending on the risk associated with the selected donor, the recipient, the host-vector relationship and the resultant GMO.

CHAPTER 2

THE RISK ANALYSIS PROCESS: BASIC CONCEPTS

Risk analysis can be broadly defined as an integrated process consisting of three major components: risk assessment, risk management and risk communication. The individual components are distinct, but are linked to achieve a well-functioning risk analysis process that forms the basis for decision-making on any operation or dealing of GMOs (Australian Government, 2005).

RISK ANALYSIS
An integrated process to analyse risk and form the basis for further decision-making.

In the case of biosafety, risk analysis involves a scientific process to estimate the risks to human life and health, as well as the impact on the environment, associated with the use of a particular GMO or its products. The prevention, reduction or elimination of these risks requires methods of risk management that are normally implemented as actions conforming to particular regulations. Risk assessment and risk management have to be implemented along with risk communication, which involves all interested parties and allows for an iterative process of risk analyses.

Risk assessment is important in the process of risk analysis given that if a particular risk is not identified, the steps taken to reduce it cannot be formulated in the risk management process. Risk assessment relies on a solid scientific base. Each case has to be dealt with individually and a separate evaluation has to be undertaken for each phase of obtaining, researching, testing, producing and releasing into

RISK ASSESSMENT
A rigorous science-driven process used to identify a hazard and obtain qualitative or quantitative estimates of the levels of risk posed by a hazard.

the environment of GMOs on a large or small scale. The complexity of the risk analysis process applied to a large variety of genes and gene combinations is very high, since this can result in a vast range of effects and interactions. In this sense, evaluation of possible impacts over the long term presents many difficulties. Moreover, the results of risk assessments from small-scale tests cannot be extrapolated to the large scale.

2.1 COMPONENTS OF RISK ANALYSIS

Risk assessment is the first and the *scientific component* of risk analysis. It is a rigorous science-driven process used to identify a hazard and obtain qualitative or quantitative estimates of the levels of risk posed by a hazard, including possible adverse effects on human health and the environment. It typically consists of four steps: (1) hazard analysis (identification and characterization), (2) likelihood estimation, (3) consequence evaluation; and (4) risk estimation. A more detailed discussion of risk assessment is presented in Chapter 3.

RISK MANAGEMENT
Is concerned with evaluating whether the risks identified by the risk assessment process are acceptable and manageable, then selecting and implementing the control measures as appropriate to ensure that risks are minimized or controlled.

Risk management is the second and *decision-making component* of the process of risk analysis. It is primarily supported by risk assessment but is also supported by other risk considerations. Risk management is concerned with evaluating whether the risks identified by the risk assessment process are acceptable and manageable, then selecting and implementing the control measures as appropriate to ensure that risks are minimized or controlled. A more detailed discussion on the methodology of risk management and other considerations is presented in Chapter 4.

RISK COMMUNICATION
The process of exchange of information and opinions concerning risk and risk-related factors among various stakeholders concerned with risk.

Risk communication is recognized as the third component that underpins the risk assessment and risk management processes. It is the process of exchange of information and opinions concerning risk and risk-related factors among various stakeholders concerned with risk (Codex Alimentarius Commission, 2003). It strengthens the overall process of risk analysis by helping to define the issues and

providing the link and the feedback mechanism that informs the two processes of risk assessment and risk management (FAO, 1999). The principles, structures and processes of risk communication are presented in Chapter 5.

The interplay between risk assessment, risk management and risk communication is depicted in Figure 2.1:

Figure 2.1 | **Generic components of risk analysis**

Adapted from: FAO, 2007.

Risk analysis applied in the broad sense separates the risk assessment from risk management. The reasons are: to maintain the scientific integrity of the risk assessment, to avoid confusion over the functions to be performed by risk assessors and risk managers, and to minimize any conflict of interest. In practice, however, this separation is rarely clear-cut and variation in its implementation exists among countries and across regulatory institutions.

2.2 PRINCIPLES OF RISK ANALYSIS: GENERAL ASPECTS

PRINCIPLES OF RISK ANALYSIS
General aspects of risk analysis have been defined that need to be maintained to assure reliability of the obtained results.

While regulatory frameworks for risk analysis vary among countries, the underlying general principles in assessing risks posed by GMOs to human health and the environment share many similarities. These include:

Science-based – Risk should be assessed using information obtained through application of science and scientific methods, i.e. rigorous and systematic, reproducible, with testable null hypothesis, qualitative and/or quantitative. Methods used should be appropriate and data generated of high quality to withstand scientific scrutiny and peer review.

Open, transparent and documented – All aspects of the process of risk analysis should be fully documented in a transparent manner. Documentation should be accessible to all interested parties, while respecting legitimate concerns to preserve confidentiality. This principle also refers to the selection of experts who will conduct the risk assessment. Experts responsible for risk assessment should be selected on the basis of their expertise, experience, and their independence with regard to the interests involved.

Case by case - Risk should be assessed on a case-by-case basis. This means that for each case, the risk assessment methodology and required information may vary in nature and level of detail, depending on the GMO concerned, its intended use (e.g. laboratory, field, market) and the likely potential of the receiving environment (e.g. presence of wild relatives, non-target species, endangered species, etc.).

Comparative - Risks should be compared with background risks, i.e. risks are considered in the context of the risks posed by the non-modified recipients or parental organisms, within the context of the intended use. This requires appropriate comparators and well-established baseline information.

Systematic - The risk analysis should follow a structured, step-by-step approach. The key steps are: establish the purpose, scope and boundaries of the risk assessment, assess the risk, and manage and communicate the risks.

Iterative - Risks should be evaluated and reviewed as appropriate in the light of newly generated scientific data. Conclusions and assumptions should be examined relative to new information.

Inclusive – The process of risk analysis should be all-encompassing. The three components of risk analysis should be applied within an overarching framework for management of food- or organism-related risks to human health and the environment. It should draw information from a wide range of credible sources and could also take into account expert advice of, and guidelines developed by, relevant international organizations. Effective communication and consultation with all interested parties should be ensured in all aspects and stages of the process of risk analysis.

2.3 THE METHODOLOGY OF RISK ASSESSMENT AND RISK MANAGEMENT: KEY STEPS

METHODOLOGY OF RISK ANALYSIS
Key steps of the process include: hazard analysis, likelihood estimation, consequence estimation, risk estimation, and risk management.

General guidance on the **methodology of risk assessment and risk management of GMOs** exists and they share many similarities. Annex III 8 of the Cartagena Protocol on Biosafety (CBD, 2000) is a good exemplary guide and the steps typically followed are enumerated below.

- Hazard analysis - An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health.
- Likelihood estimation - An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism.

- Consequence evaluation - An evaluation of the consequences should these adverse effects be realized.
- Risk estimation - An estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized.
- Risk management – A recommendation as to whether or not the overall risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks, including monitoring.

It should be noted that the level of details and sequence of some of the steps indicated above vary across countries. More detailed discussions of the methodology of risk assessment and risk management are presented in later chapters (see Chapters 3 and 4).

2.4 CONCEPTS AND ISSUES IN RISK ANALYSIS

There are a number of concepts and issues that are very important in gaining a better understanding of the process of risk analysis. These include:

2.4.1 The concept of familiarity

FAMILIARITY
Evaluating the potential risks of a GMO by comparing it with its non-modified counterpart.

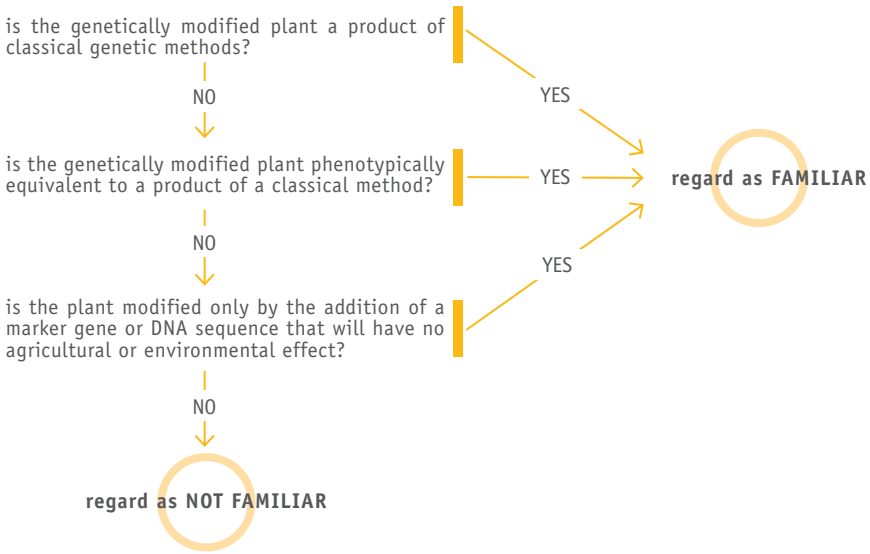
Risk assessment of GMOs requires information on the identity, characteristics and history of safe use of the organism that is subjected to genetic modification. Most GMOs to date have been developed from organisms that are "familiar" i.e. there is sufficient information available about the organism's attributes, and a long history and experience of its safe use.

The concept of familiarity provides a way to recognize the potential risks by using already available information on the attributes of the organism. Because of familiarity, effective methods can de devised to avoid or manage the risks

to acceptable levels. For example, it is possible to determine the potential for invasiveness of a GM crop based on knowledge of the biology of the non-modified organism (e.g. presence of traits that are associated with invasiveness) and the presence of wild compatible relatives. Likewise, it is possible to identify the potential allergenicity of a GMO if knowledge and history of safe use of the origin/source of the gene used in genetic modification is available. In this context, the concept of familiarity is not a risk assessment in itself, but a useful tool for identifying, evaluating and managing risks.

An example of a familiarity test for genetically modified plants is shown in the following illustration (Persley *et al.*, 1993):

Figure 2.2 | **A familiarity assessment framework**



2.4.2 The concept of substantial equivalence

SUBSTANTIAL EQUIVALENCE
The principle that GMOs can be compared with their conventional counterparts that have an established history of safe use.

In assessing the risks posed by GMOs to human health and the environment, the concept of familiarity is used together with the concept of **substantial equivalence**. Substantial equivalence is based on the principle that GMOs can be compared with their **conventional counterparts** that have an established history of safe use (Codex Alimentarius Commission, 2003). The concept is used to identify the similarities and differences (including intended changes and unintended changes) between the GMO and its conventional counterpart to be able to determine if the GMO is "as safe as" or presents any new or greater risks than its conventional counterpart. The concept of substantial equivalence does not establish absolute levels of safety, but relative levels of safety.

CONVENTIONAL COUNTERPART
A related organism/variety of the GMO, its components and/or products for which there is experience of safety based on common use as food.

Internationally, the concept of substantial equivalence is recognized as one of the principles for environmental risk assessment by the Cartagena Protocol on Biosafety, and in food safety assessment by the Codex Alimentarius Commission. The relevant texts (in italics) are as follows:

Cartagena Protocol on Biosafety (CBD, 2000)

Annex III 5 – Risk Assessment

Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, *should be considered in the context of the risks posed by the non-modified recipients or parental organisms* in the likely potential receiving environment.

Codex Alimentarius Commission Principles and Guidelines on Foods Derived from Biotechnology (Codex Alimentarius Commission, 2004), Section 3.10 – Principles:

> Risk assessment includes a safety assessment (…) The safety assessment *should include a comparison between the food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences*. If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health.

It should be noted that the concept of substantial equivalence is considered a *starting* point for the safety assessment to structure the safety assessment procedure, and to focus on the identified differences that may require further testing. Its application is limited by the choice of an appropriate comparator and availability of sufficient scientific information relevant to the risk assessment. These points are illustrated in the three cases presented below.

- *GMOs that are shown to be substantially equivalent to the conventional counterparts* are regarded as being "as safe as" their counterpart. No further safety considerations other than those for the counterpart are necessary.
- *GMOs that are substantially equivalent to the conventional counterpart except for defined differences* need further safety assessment which should focus only on the defined differences. Typically, the defined differences will result from the intended effect of the genetic modification that may, or may not, change the endogenous traits, or produce new traits in the host organism.
- *GMOs that are not substantially equivalent to the conventional counterpart*. Up to now, and probably for the near future, there have been few examples of these GMOs. Nevertheless, it is conceivable that with future developments in biotechnology, these kinds of GMOs will be produced. In these cases, the concept of substantial equivalence cannot be applied.

As a final note, in addition to the limitations mentioned above, the use of the concept of substantial equivalence in risk assessment has been criticized as subjective, inconsistent and pseudo-scientific (Millstone *et al*. 1999). However,

despite its limitations and criticisms, there is wide recognition that the concept of substantial equivalence remains the most practical approach currently available to framing the risk assessment process.

2.4.3 The precautionary approach

THE PRECAUTIONARY APPROACH
"Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

Principle 15 of the Rio Declaration on Environment and Development (UNCED, 1992) states that:

"In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

There are a number of important points to keep in mind about Principle 15 of the Rio Declaration in conducting risk analysis.

- The term "precautionary approach" is specifically used to differentiate it from the legal connotation of the term "precautionary principle". The latter is compulsory or legally binding while the former may be binding in some cases but normally does not have the same force as a law (Recuerda, 2008). Because it is an "approach" and not a "principle", Principle 15 allows for discrimination between countries in applying the approach based on their capability, which a law or principle will not allow. Furthermore, Principle 15 allows other costs (e.g. social or economic) to be considered in order to be cost-effective in applying the approach. In view of these, the "precautionary approach" is viewed as softening of the "precautionary principle".
- The precautionary approach in the context of Principle 15 explains the idea that scientific uncertainty (i.e. source or form of doubt) should not prohibit using preventive measures to protect the environment; and use of "cost-effective" measures indicates that costs can be considered when applying the approach.

- Principle 15 identifies the triggers to propose a precautionary approach.
- Finally, Principle 15 refers to potentially irreversible harm to be the most important application of the precautionary approach. Where risks for irreversible damage is high, decision-makers will act from the perspectives of prudence and precaution.

Many countries have adopted the same phrasing of Principle 15 of the Rio Declaration in their regulatory systems and have established risk assessment mechanisms based on the precautionary approach. The interpretation and implementation of the precautionary approach vary across countries because they differ in their opinions on thresholds of risk and degree of scientific uncertainty allowed in the process of risk analysis. Many regulatory approaches recognize the imperfect nature of evidence when making decisions. In conformity with the precautionary approach, preventive measures are built in their risk management design to allow certain activities with limitations, when appropriate.

2.4.4 Uncertainty

UNCERTAINTY
An inherent property of risk and present in all aspects of risk analysis, including risk assessment, risk management and risk communication.

Uncertainty is an inherent property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication (Hayes, 2001). Simply defined, uncertainty is a form of source of doubt. There are five different types of uncertainty that can be applied to risk analysis, which are enumerated below:

- *epistemic* - uncertainty of knowledge, its acquisition and validation. The most common examples are statistical errors, use of surrogate data (e.g. extrapolation from animal models to humans), and incomplete, ambiguous, contested or unreliable data. Epistemic uncertainty could be reduced by designing more rigorous experiments, and by applying more powerful statistical analyses and GLP.
- *descriptive* - uncertainty of descriptions that may be in the form of words (linguistic uncertainty), models, figures, pictures or symbols (such as those used

in formal logic, geometry and mathematics). Usually associated with qualitative measurements and inconsistent and incomplete definition and application of words. For example, the word "low" may be ambiguously applied to likelihood of harm, magnitude of a harmful outcome and to the overall estimate of risk. Descriptive uncertainty could be reduced by using accurate and consistent definitions and providing clear parameters, scope and boundaries.

- *cognitive* (including bias, perception and sensory uncertainty) - cognitive uncertainty can be viewed as guesswork, speculation, wishful thinking, arbitrariness, doubt, or changeability. One way to reduce cognitive uncertainty is through effective communication strategies.
- *entropic (complexity)* - uncertainty that is associated with the complex nature of dynamic systems such as a cell, an organism, the ecosystem, or physical systems (e.g. the atmosphere). Complexity and incomplete knowledge contribute to the inability to establish the complete causal pathways in a system. Consequently, a deterministic system can have unpredictable outcomes because the initial conditions cannot be perfectly specified. Complexity could be reduced by generating more information about the various components and relationships in the system.
- *intrinsic* - uncertainty that expresses the inherent randomness, variability or indeterminacy of a thing, quality or process. Randomness can arise, for example, from genetic difference. A critical feature of intrinsic uncertainty is that it cannot be reduced by more effort, such as more data or more accurate data. In risk management, safety factors and other protective measures are used to cover this type of uncertainty.

There are a number of ways to address uncertainty in risk analysis of GMOs:

- Request or obtain further information on the specific issues of concern. Where there is uncertainty, more experiments may be required in order to answer the question. However, it must be recognized that the effort and resources required to acquire greater knowledge increase almost exponentially with each demand

BOX 2.1

EXAMPLES OF UNCERTAINTY WITHIN THE ELEMENTS OF RISK ANALYSIS

Risk assessment

- Uncertainty in the nature of the GMO, such as the lack of knowledge of biochemical properties of the introduced genes, environment-specific performance of the GMO, its interaction with other biological entities and processes, or landscape changes over long time periods;
- Uncertainty of the calculations within the risk assessment process, including assessment of hazards, likelihood and consequences;
- Uncertainty in descriptions used in qualitative risk assessments due to insufficient explanations of terminology, use of related terms that are not fully congruent or the use of the same term in different contexts.

Risk management

- Balancing the sufficiency of protective measures against their effectiveness;
- Decision-making in the presence of incomplete knowledge and conflicting values.

Risk communication

- Uncertainty of communication effectiveness due to difference in knowledge, language, culture, traditions, morals, values and beliefs.

Adapted from: Australian Government, 2005.

for greater precision or detail. In many instances, these may not be technically (e.g. no valid protocol) or practically (e.g. unaffordable cost) possible.

- Implement appropriate risk management strategies and/or monitor the GMO in the receiving environment.
- In cases where further experimentation may not provide the necessary information, the "worst case" scenario approach can be applied, where the focus is less on determining the likelihood of an occurrence, but rather on evaluating what the consequences of the occurrence would be.

CHAPTER 3

THE RISK ANALYSIS PROCESS: RISK ASSESSMENT

COMPONENTS OF RISK ASSESSMENT
(1) hazard analysis (hazard identification and characterization), (2) likelihood estimation, (3) consequence evaluation; and (4) risk estimation.

Risk assessment is the core of biosafety because it represents the basis for making decisions on the protection of the environment and human health in the case of uncertain scientific backgrounds. To guarantee its integrity and objectivity, risk assessment has to be separated from risk management.

Risk assessment is a science-based process consisting of four steps: (1) hazard analysis (hazard identification and characterization), (2) likelihood estimation, (3) consequence evaluation; and (4) risk estimation, all of which are described below.

A generally accepted methodology for biotechnology risk assessment has been outlined in several easily accessible documents including the UNEP International Technical Guidelines for Safety in Biotechnology (UNEP, 1995), the EC Directive 2001/18/EEC, and Annex III 8 (a-d) of the Cartagena Protocol on Biosafey (CBD, 2000). In this section, the latter is used as a guide to enumerate the steps typically

followed in risk assessment whether for food products or organisms released into the environment. The additional information to help explain each step was abstracted primarily from the Risk Analysis Framework of the Australian Government (2005) and the FAO Biosecurity Toolkit (2007).

3.1 KEY STEPS IN RISK ASSESSMENT

3.1.1 Hazard analysis, identification and characterization

HAZARD ANALYSIS, IDENTIFICATION AND CHARACTERIZATION
An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment.

Hazard analysis can be defined as an identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health (CPB, Annex III 8 (a)).

Hazard identification investigates the intrinsic or "built-in" potential of the biological agent (e.g. GMO or GM foods) to cause harm. Hazard characterization aims to evaluate, in qualitative and quantitative terms, the nature of the identified intrinsic hazard. Quantitative and qualitative techniques are used in hazard identification (Hayes *et al*, 2001). Qualitative techniques include checklist, brainstorming, expert consultation, fault and event trees. Quantitative techniques include HAZOP analysis, hierarchical holographic model (HHM), SWOT analysis, Delphi analysis, etc. Approaches to hazard analysis may be inductive (top down) or deductive (bottom up). A checklist and the inductive approach appear to be the status quo of hazard analysis. Evidentiary support could range from unsubstantiated statements (weak evidence) to experimental data (strong evidence).

Hazard analysis also involves establishing the causal link and pathway or route of exposure between a hazard and an adverse outcome. It also involves identifying the measurable properties of the hazard in order to accurately assess that harm has occurred. Table 3.1 summarizes examples of potential biological harms and the respective measureable properties.

Table 3.1 | **Examples of potential harms and their measurable properties**

Hazard	Measurement Attributes
Increased fitness, increased persistence, invasion of a GMO	Occurrence and biological properties – traits for weediness and invasiveness
Toxicity of a GMO to non-target organisms	Mortality; survival; population morbidity, species richness
Habitat modification - altered bio/geo-chemical cycles	Carbon, nitrogen, phosphorus flux; frequency of floods, fire; pollutant concentration
Loss of biodiversity and extinction of species	Diversity indices; species richness
Creation of new viruses	Occurrence, number, severity, host range
Human toxicity and allergenicity	Biological, physiological and physical abnormalities; mortality; frequency and age of morbidity

3.1.2 Likelihood estimation

LIKELIHOOD ESTIMATION
An evaluation of the likelihood of adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism.

Likelihood estimation can be defined as an evaluation of the likelihood of adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism (CPB, Annex III 8 (b)).

Likelihood is the probability that the harm will occur. It is expressed as a relative measure of frequency (the number of occurrences per unit time) and probability (from zero to one, where zero is an impossible outcome and one is a certain outcome). It is important to remember that likelihood estimation is a predictive process. The accuracy of prediction is directly proportional to time of occurrence, i.e. a short-term outcome is more accurately assessed than a long-term outcome.

Here the term “estimation” is chosen because exact numbers of the frequency with which something will happen in nature cannot always be measured or predicted. It is possible in certain risk calculations, such as non-target risks, but more frequently the risk finding is qualitative on the basis of a weight of evidence analysis.

Likelihood assessment may be qualitatively described as follows:

- *Highly likely* - is expected to occur in most circumstances
- *Likely* - could occur in many circumstances
- *Unlikely* - could occur in some circumstances
- *Highly unlikely* (negligible or effectively zero) - may occur only in very rare circumstances

For GMOs, the most important factors that contribute to the likelihood that harm will occur are the survival, reproduction and persistence rates of the GMO, and the characteristics of the receiving environment, including its biotic and abiotic attributes.

3.1.3 Consequence evaluation

CONSEQUENCE EVALUATION
An evaluation of the consequences should adverse effects be realized.

Consequence evaluation is an evaluation of the consequences should adverse effects be realized (CPB, Annex III 8 (c)). Consequence evaluation involves characterizing the significance and impact of the adverse outcome if the hazard occurs. The following criteria should be taken into consideration:

- severity – number, magnitude, scale;
- spatial extent – geographical (local, national, global); organism (individual, population, community, ecosystem);
- temporal extent – duration and frequency;
- cumulation and reversibility;
- background risk – risk that may occur in the absence of the stressor (e.g. GMO).

Descriptors of consequence assessment:

- *Marginal* **-** minimal or no injury except to a few individuals who may require medical aid; minimal or no degradation of the environment;
- *Minor* **-** slight injury to some people who may require medical treatment;

disruption to biological communities that is reversible and limited in time and space or number of individuals/populations affected;

- *Intermediate* - injury to some people who require significant medical treatment; disruption to biological communities that is widespread but reversible or of limited severity;
- *Major* - Severe injury to some people who may require hospitalization or may result in death; extensive biological and physical disruption of whole ecosystems, communities or an entire species that persists over time or is not readily reversible.

3.1.4 Risk estimation

RISK ESTIMATION
An estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized.

Risk estimation is an estimation of the risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized (CPB, Annex III 8 (d)).

Risk estimation combines the information on likelihood and consequence of the identified hazard to come up with the risk estimate matrix shown below (Figure 3.1). As a general rule, risks with moderate and high estimates will invoke the corresponding risk management treatments or control measures.

Descriptors of risk estimate:

- *Negligible* - risk is insubstantial and there is no present need to invoke actions for mitigation;
- *Low* - risk is minimal, but may invoke actions for mitigation beyond normal practices;
- *Moderate* - risk is of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective;
- *High* - risk is unacceptable unless actions for mitigation are highly feasible and effective.

Figure 3.1 | **The combinations between severity and probability of a hazard and the resulting risk level classification**

			PROBABILITY				
			frequent	likely	occasional	seldom	unlikely
			A	B	C	D	E
SEVERITY	catastophic	I	extremely high				
	critical	II		high			
	moderate	III		medium			
	negligible	IV		low			
			RISK LEVEL				

Adapted from: Australian Government, 2005.

Finally, in conducting the steps outlined above, the following characteristics, depending on the dealing of GMOs, could be taken into consideration:

- recipient, host or parental organisms;
- inserted genes, sequences and related information about the donor(s) and the transformation system;
- the resulting GMO;
- available methods for detection and identification of the GMO;
- the intended use (e.g. the scale of the activity - field trial or commercial use);
- the receiving environment.

3.2 INFORMATION REQUIREMENTS FOR RISK ASSESSMENT

Risk assessment for the release of GMOs typically takes into consideration the points enumerated above obtained from Annex 9 of the Cartagena Protocol on Biosafety. A more detailed discussion of the various points is presented below (Konig *et al.*, 2002).

3.2.1 Information on the recipient or parent organism

INFORMATION ON THE RECIPIENT OR PARENT ORGANISM
Includes identity, agronomic performance, geographic distribution, history of safe use, compositional analysis, etc.

The type of information on the parent crop that should be gathered at the outset include:

- *Identity, phenotypic and agronomic performance* – taxonomic identity (including the complete name, family name, genus, species, subspecies, cultivar/breed/race/isolate, common name, and sexually compatible wild relatives); chemical proximate composition and key nutrients and anti-nutrients.
- *Geographical distribution/source or origin* – area of cultivation, centre of origin and centre of diversity.
- *History of safe use* – any known nutritional, antinutritional, toxicological, allergenic characteristics or intolerances; importance in the diet, including information on preparation, processing, and cooking.
- *Compositional analysis* – key nutrients, toxins, allergens, antinutrients, biologically active substances associated with parent and sexually compatible relatives; information both from the literature and from analytical data.

The recipient or parent organism refers to the organism into which the genes are introduced through genetic modification methods. The characteristics of the recipient organism guide the choice of test parameters for comparison of the GMO with its non-modified counterpart, i.e. it serves as a reference point. Knowledge of the natural variation of the traits in the recipient is essential in interpreting data when comparing the GMO with its non-modified counterpart under different receiving environments. The history of safe use of the parent can provide additional information to help plan the risk assessment strategy, e.g. identifying what should be the focus of further assessments.

The OECD has been compiling consensus documents (OECD, 2009) on the (1) biological attributes and (2) compositional characteristics for certain crop species. These documents provide excellent sources of relevant information on

the parent or recipient crop. Information from these OECD consensus documents has been accepted by biosafety regulatory authorities in some countries.

3.2.2 Information on the inserted genes and sequences and related information about the donor(s) and the transformation system

INFORMATION ON THE INSERTED GENES AND SEQUENCES
Includes description of the donor, description of the vector, characterization of the inserted DNA and the insertion site.

The information required includes:

- *Description of donor(s)* – includes classification and taxonomy, evidence of potential toxicity, allergenicity or pathogenicity, history of use and exposure to the donor; and, where possible, function of any recombinant DNA sequences used in the transformation.
- *Description of vector DNA* – includes information on the source of all genetic elements used to construct and amplify the transformation vector, including coding sequences, promoters and termination signals, vector maps with relevant restriction sites; proof of absence of vector fragments not intended to be transferred, and nucleotide sequence information.
- *Transgene delivery process* – For *Agrobacterium*-mediated transformation the information requirement includes donor strain and any plasmid contained in that strain; for direct transformation methods, such as the particle gun, it includes proof of absence of contaminating sequences of bacterial chromosomal DNA or other plasmid DNA or vector sequences.
- *Characterization of introduced DNA* – includes information on the number of insertion sites, copy number of the introduced DNA, ends of inserts adjacent to host genomic DNA; a genomic library of each transformed plant line (under discussion), absence of vector backbone; and verification of the stability of transgene insertion over five or more generations.
- *Characterization of insertion site* – information on the junction of the inserted recombinant DNA and the host genome.

With regard to the transformation method, it has been argued that in using *Agrobacterium,* the risk of transfer of random DNA to the plant is relatively small (Gelvin, 2000). The vector with the recombinant DNA may be separate from the vector with transfer function and contain a recognition site for the transfer-mediating gene products, thus limiting the chance of transferring transfer vector DNA.

With regard to the characteristics of the introduced DNA, all inserted *functional genes* are, in principle, relevant to the risk assessment, regardless of whether they are the "genes of interest" or genes that have "travelled along" in the process, such as selectable marker genes. The underlying reason is the possibility of unintended effects due to the presence of these DNA sequences. For example, a gene with a prokaryotic origin of replication (*ori*) will not be expressed in a plant cell, but will be considered in the risk assessment because it may facilitate replication of the gene in the – unlikely – event that it is taken up and recovered in a bacterium. To conclude, all regulatory regions and other sequences that are transferred to an organism in addition to the functional genes need to be included in the risk assessment.

Finally, the level of detail required should depend on the nature of the dealing. For example, in the early stages of research and development of the GM product, when full molecular characterization has not yet been conducted, it can be assumed that the entire construct may have been integrated into the recipient organism. Hence, the risk assessment is conducted on that basis and risk is managed by strict containment measures (see Section 2). When the activity has moved on to confined field trials, more detailed characterization is requested, leading to a full characterization as required for large-scale field trials or commercial/ market release. This is part of the "case-by-case" and "step-by-step" approach of risk analysis.

3.2.3 Information on the gene products; recombinant proteins and/or metabolites

INFORMATION ON THE GENE PRODUCTS
Includes characterization of proteins, mode of action, toxicity, allerginicity, etc.

With certain exceptions, like anti-sense DNA, all inserted functional genes transferred to the recipient organism are translated into primary (protein) and secondary (metabolite) gene products. Hence, both are relevant to the risk assessment process. The information required for the gene products is:

- *Structure, identity and characterization* – For proteins, this includes the molecular weight, amino acid sequence, post-translational modification (e.g. level of glycosylation and phosphorylation), immuno-equivalence, activity and specificity of catalysed reactions (if the gene product is an enzyme), expression levels (recombinant protein levels in various host tissues), changes in levels of inherent crop micro or macronutrients (e.g. Vitamin A in Golden Rice), and significant unexpected changes in the levels of substances detected during compositional analysis.
- *Mode of action/specificity* - mechanism of action (e.g. Bt-proteins which are toxic to certain insects but not humans), overview of all relevant metabolic pathways that could be affected by the enzyme's presence or altered levels or substance specificity (e.g. the CP4 EPSPS enzyme that confers tolerance to the herbicide glyphosate but does not affect the biosynthesis of the aromatic amino acids of all plants and micro-organisms).
- *Toxicity* – information on documented exposure and history of safe use; results of previous toxicity testing programmes; for novel proteins/metabolites, information on structure and function and toxicity tests are required.
- *Allergenicity* – changes in the characteristics or levels of expression of endogenous allergenic proteins, and/or allergenicity of the recombinant protein itself.

TOXICITY AND ALLERGINICITY
The primary concerns and focus of risk assessment, particularly for GMOs that will be used as food/feed.

Toxicity and allergenicity of the gene products are the primary concerns and focus of risk assessment, particularly for GMOs that will be used as food/feed. From the perspective of food/feed safety, it is widely recognized that proteins are not generally toxic when consumed orally as they are largely part of a standard human and animal diet. However, almost all allergens are proteins. With regard to toxicity, safety concerns

and the amount of new data that will be required should be carefully considered in the light of existing information on the protein/metabolite prevalence, similarity to proteins/metabolites that are routinely used by humans and animals, and history of exposure. Safety concerns and new data requirements should be lower in the case of proteins that have no history of adverse effects on humans and animals. With regard to allergenicity, the amount of new data required should take into account the following key considerations: (a) Is the recombinant protein derived from an allergenic source or known allergen? Is it able to induce *de novo* sensitization?; Is it cross-reactive with IgE antibodies raised by known allergens?; (b) Has transformation altered the allergenic properties of the product derived from the GMO?

3.2.4 Information on the resulting GMO

INFORMATION ON THE RESULTING GMO
Includes identity, agronomic analysis, compositional analysis, and safety analysis.

Information requirement for the resulting GMO includes:
(1) identity, phenotypic and agronomic analysis;
(2) compositional analysis and
(3) safety analysis (animal studies). The information from these analyses is obtained in comparison with the non-GM counterpart. These analyses focus on detecting any indicative differences in test parameters, such as agronomic performance, compositional and nutritional values, and dietary subchronic responses in animal feeding studies.

Sources of data to enable detailed comparison can come from a variety of sources. Data about the resulting GMO are available from growing the GMO in growth chambers, greenhouses and/or earlier field trials. Field trials are usually undertaken under a diversity of environmental conditions representative of those typical for planned commercial growing. Other major sources of data are databases on existing food composition, chemical analyses, and toxicology tests. Data can also be obtained from the Biosafety Clearing House for information on field and commercial releases of identical GMOs in various locations.

GMO Detection and identification methods are important in hazard identification and characterization. In various stages of research, development and release of a GMO, molecular characterization and toxicological tests are conducted to generate information on the characteristics of the inserted DNA sequences, the gene products, and the resulting GMO. This means that detection, identification and test methods focusing on the inserted DNA, the resulting proteins and the resulting GMO are crucial for GMO analysis.

GMO DETECTION AND IDENTIFICATION METHODS
Highly important for hazard identification and characterization; described in detail in Module A.

Examples of currently available DNA-based GMO detection methods widely used include:

- Southern blot
- Qualitative PCR
- Quantitative real-time PCR
- DNA chips

Protein-based testing methods include:

- Western blot
- ELISA
- Lateral flow strips
- Protein chips

Toxicology test methods include:

- i*n vivo* and *in vitro* test systems
- chronic toxicity, carcinogenicity and reproduction studies
- acute animal toxicity studies

Each of these methods has its own advantages and disadvantages in terms of targets, ease of use, specificity, sensitivity, costs, etc. Existing methods have proven to be adequate for the safety assessment of the GMOs that are currently available on the market. Development in the areas of detection and testing are being pursued

to improve existing techniques and address the safety of next generation products of modern biotechnology.

For a more detailed discussion on DNA and protein detection techniques, please refer to Module 1: Agricultural Biotechnology.

3.2.5 Information relating to the intended use of a GMO

INFORMATION RELATING TO THE INTENDED USE OF A GMO
This may include a wide range of activities, from basic research to large-scale commercial release.

The intended use of a GMO possibly encompasses a wide range of activities and applications. These include: (a) make, develop, produce or manufacture GMOs; (b) conduct experiments with GMOs; (c) breed GMOs; (d) propagate GMOs; (e) investigate the use of GMOs in the course of development or manufacture of a product; and (f) grow, raise or culture GMOs, possibly on an industrial scale.

These activities and applications can be classified into two categories: (1) contained use; and (2) release into the environment. Contained use means any operation undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment (CPB definition).

Release into the environment, in this document, refers to non-contained usage of GMOs. In many regulatory systems, this means any trial conducted in the field irrespective of scale and availability of confinement measures and commercial release. The major distinction between commercial release and field trials is that with field trials the GMO involved is still under various degrees of control, whereas after placing the GMO on the market for commercial production, its use is, in principle, unrestricted except for specific product-use conditions, such as labelling or monitoring.

3.2.6 Information on the receiving environment

INFORMATION ON THE RECEIVING ENVIRONMENT
The characteristics of the receiving environment are crucial for the risk assessment.

The characteristics of the receiving environment are crucial for the risk assessment. When releasing genetically modified plants into the environment, there are relevant questions for specific applications to be assessed:

1. Is there potential for negative impact on managed ecosystems?
2. Does the GMO have altered resistance to insects or pathogens?
3. Does the GMO have new weed characteristics?
4. Does the GMO pose hazards to local fauna or flora?
5. Is there potential for negative impact on natural (non-managed) ecosystems?
6. Are cross-hybridizing relatives present in the same area?
7. Can the new trait impart increased competitiveness to weedy relatives?
8. Does the GMO have new weed characteristics that could make it successful outside of the managed ecosystems?

For field trials, the information requirement includes the specific physical location of the trial, taking into consideration the following relevant characteristics:

- comparison between the normal growing environment with the proposed environment for release;
- specific environmental factors influencing survival and distribution of the organism (e.g. climate, soil conditions);
- presence of sexually compatible crops;
- presence of sexually compatible wild relatives.

Taken together, it should be clear that risk assessment is a complex, science-driven process, that needs to integrate a variety of data and considerations. Since every GMO is different concerning its design, purpose, biology of the parent organism and the likely receiving environment, risk assessment has to be performed on a case-by-case basis for each individual GMO case. In Annex 4, a summary of points to be taken into consideration for the risk assessment of GMOs, extracted from European Community (EC) legislation, is provided as an additional guideline.

CHAPTER 4

THE RISK ANALYSIS PROCESS: RISK MANAGEMENT

RISK MANAGEMENT
The process of weighing policy alternatives to mitigate risks in the light of risk assessment, and, if required, selecting and implementing appropriate control options, including regulatory measures.

Risk management is the second and the decision-making component of the process of risk analysis. **Risk management** is defined as "the process of weighing policy alternatives to mitigate risks in the light of risk assessment, and, if required, selecting and implementing appropriate control options, including regulatory measures" (FAO/WHO, 1995). Its objective is to determine which risks require management and how these risks can be effectively managed or controlled so that the goal of ensuring adequate protection for people and the environment is attained.

The management of risk is basically founded on:

- Understanding and identification of risks and adverse conditions associated with work, which are determined in the risk assessment process. The principal objective of the evaluation is to know which management measures and controls are to be applied to the identified risks. If a risk is not identified, one cannot develop risk management procedures.
- The development and implementation of technical and organizational measures that correspond with the determined risks.
- The type of organism released (transgenic, non-transgenic, exotic).

The risk management framework is depicted in Figure 4.1:

Figure 4.1 | **Components of a generic risk management framework**

Adapted from: FAO, 2007.

The fundamental **objective of the risk management** process is to:

- eliminate, reduce or substitute the risk factors identified in the risk assessment;
- avoid or reduce exposure to the identified risk factors.

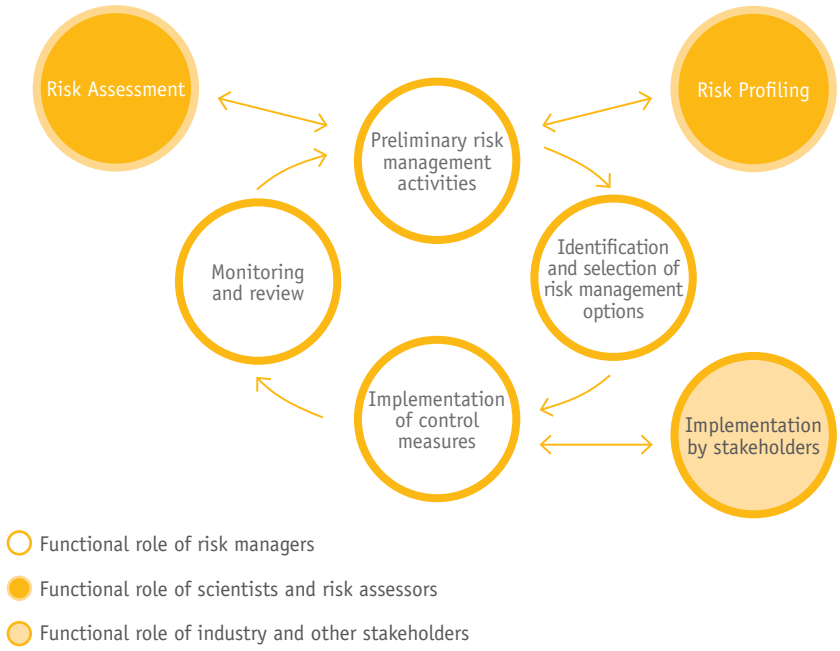
OBJECTIVE OF RISK MANAGEMENT
Eliminate, reduce or substitute the risk factors identified in the risk assessment; and avoid or reduce exposure to the identified risk factors.

As such, the measures to develop could be those for:

- elimination of risks;
- reduction of risks;
- substitution of risks.

Although other stakeholders participate in risk analysis, at a national level it is the competent authority having jurisdictional power that makes the final risk management decisions and has the overall responsibility for ensuring that control measures are properly implemented and complied with. The relations between risk managers, scientists and risk assessors and other stakeholders is depicted in Figure 4.2:

Figure 4.2 | **Role of the risk manager in application of the generic risk management process**



Adapted from: FAO, 2007.

The preliminary risk management activities can indicate that:

- The available information is sufficient and the competent authority can therefore be authorized to evaluate the risks.
- The available information is not adequate and it is therefore necessary to request more information.
- The case is straightforward or there already exists adequate experience and information on risks and the required biosafety measures, and the formal risk evaluation can begin.

If the risk evaluation is commissioned, the competent authority should clearly and objectively formulate the scope of the risk evaluation and the questions to be addressed.

Risk management measures are various; they can be simple or a combination of different measures, contributing to more complex management. The general procedures are those applicable to all organisms (transgenic or non-transgenic) before their use and release into the environment according to technical and engineering measures, including control techniques and organizational measures. Specific measures depend on the type of organism.

In any case, risk management is primarily supported by the results of the risk assessment process but may consider risks in a wider context. This allows the risk manager or designated national competent authorities to take into consideration other inputs, e.g. socio-economic considerations (if allowed by the regulation) from other interested parties concerned with risks, in the final decision on any dealing of GMOs. This adds a political component to the risk management process.

There is a general consensus that, in order to maintain the scientific integrity of the risk assessment process, it is important to keep the conceptual separation between risk assessment and risk management.

KEY STEPS IN RISK MANAGEMENT
Include risk evaluation, risk mitigation, and implementing appropriate actions.

4.1 THE KEY STEPS IN RISK MANAGEMENT

Risk management is a step-by-step process which consists of:

4.1.1 Risk evaluation

In this step, decisions are made on whether the identified risk is manageable, i.e. a consideration of appropriate risk management strategies.

RISK EVALUATION
In this step, decisions are made on whether the identified risk is manageable, i.e. a consideration of appropriate risk management strategies.

The rigorous scientific process of the risk assessment implementation ends in a risk estimate. Risk evaluation starts from the result of the risk estimation step. In cases where, on the basis of the risk estimation step, the risks involved are not deemed to be "negligible" or "marginal", the risk evaluation considers whether the identified risk is manageable or acceptable. The question to address is whether the identified risks require specific risk management measures. If the answer is "yes", then a risk management strategy is defined in the next step. For example, risks with estimates of high or moderate would generally invoke a requirement for management.

Risk evaluation serves as the vital link between risk assessment and risk management. In practice, the functional separation between risk management and risk assessment is less clear in this step.

4.1.2 Risk mitigation

RISK MITIGATION
Determines the options and plans to reduce or avoid the risks.

This step is central to the risk management process. It determines the options and plans to reduce or avoid the risks. For cases where a risk management strategy has been defined, the risk assessment "loops back" to the earlier steps in the risk assessment to determine whether the proposed risk management strategies sufficiently reduce the likelihood or the consequence of potential adverse effects.. This is one reason why risk assessment is often called an "iterative process". Availability of new data, derived for instance from a confined, "risk managed" field experiment, may also be a reason to revisit and possibly revise a risk assessment.

Depending on the case, risk mitigation measures or options may include:

» specifying the appropriate containment facilities and BSLs (please see Chapter 2), as well as the conditions for use, handling, storage, transport and disposal of biological material. For genetically modified plants: reproductive isolation by removal of flowers, use of isolation distances or border rows, temporal

isolation, special design features such as male sterility, and reduction of the size or duration of an application can be considered and evaluated in:

  - Controlled field trials (isolated from other cultivated areas)
  - Semi-commercial tests (contained)
  - Commercial-scale tests (under field production conditions)
- submission of contingency or emergency plans
- monitoring and surveillance
- GMO detection (for details, please see Module 1)
- labelling (voluntary or mandatory)

BOX 4.1

POST-COMMERCIALIZATION RISK MANAGEMENT THROUGH LABELLING AND MONITORING TECHNIQUES

Risk management can include the element of traceability in the case of GMOs and particularly in the case of transgenic foods. Traceability is the capacity to follow the organisms or their products in all the phases of commercialization, along the production and distribution chains, to control quality and when necessary recall materials. This is possible through labelling and monitoring techniques and can increase costs. Traceability does not only apply to GMOs, it applies to all foodstuffs.

As far as the objectives of post-commercialization are concerned, these include:

- Following the long-term effects on human health and the environment.
- Recalling products if there is a perceived risk to human health and the environment.
- Assisting control through labelling.
- Preservation of the identity of specific products.

Detailed information on all aspects of monitoring, surveillance and emergency planning are presented in Module D.

Many countries have put up their own guidelines for dealings on GMOs but there are still no internationally agreed guidelines, except for containment, on exactly how these risk management measures are designed and implemented. Efforts are under way to standardize and harmonize the guidelines on these various risk management measures.

4.1.3 Selecting and implementing the most appropriate options and actions

SELECTING AND IMPLEMENTING OPTIONS AND ACTIONS
The final decision-making process that will ultimately lead to authorization and issuance, or rejection, of the licence required for any dealing of GMOs.

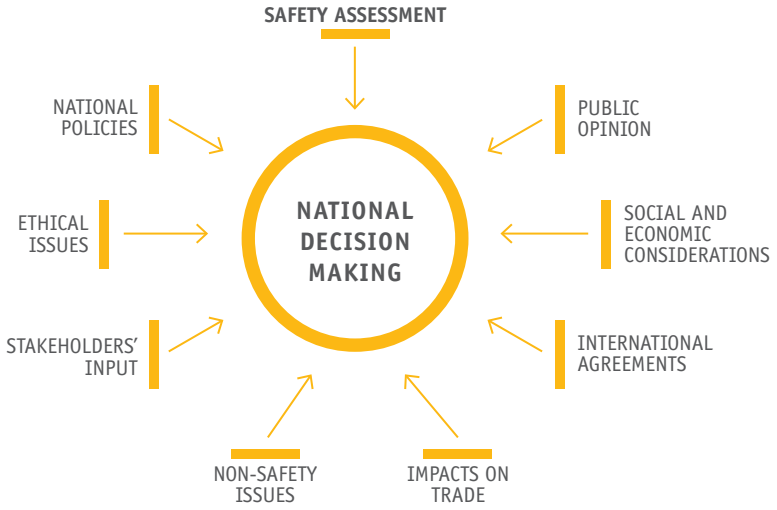
This step refers to the final decision-making process that will ultimately lead to authorization and issuance, or rejection, of the licence required for any dealing of GMOs. The risk mitigation measures identified are included as part of the licence conditions.

Final decisions are based primarily on the results of the scientific process of risk assessment. However, several factors govern decisions about the release of a GMO and in this step, the risk management process may take into account other non-risk issues (e.g. socio-economic considerations) and other risk-related factors (e.g. risk perceptions) from various stakeholders to inspire confidence and achieve wider acceptance of the decision. These stakeholders have diverse views and may have conflicting interests.

Decision-makers need to balance the individual rights of different stakeholders with the need to protect human health and the environment from the adverse effects of unacceptable risks. This step makes the risk management process essentially a political process.

Typically, decision-making incorporates, whether formally or informally, stakeholder input, public concerns and opinions, existing policies in agriculture, the environment, and food safety and responsibilities under international agreements. These factors are summarized in the following figure:

Figure 4.3 | **Factors influencing national GMO decision-making**



Adapted from: Traynor *et al.*, 2002.

Countries individually decide whether to develop, deploy, or use GMOs and the products made from them. Such decisions take into account national policies for agricultural research and development and the potential role of biotechnology in meeting national goals and objectives in food production, food security, trade and related areas. Decisions regarding the use of this technology and its products are based, in part, on a determination that they do not pose an unacceptable risk to the environment or to human health.

With the Cartagena Protocol on Biosafety, a legally binding international protocol for the safe transfer, handling and use of living modified organisms that is already in force, biosafety assessments will become part of international trade agreements.

4.2 RISK MANAGEMENT AND SOCIO-ECONOMIC CONSIDERATIONS

SOCIO-ECONOMIC CONSIDERATIONS
Such considerations might be taken into account during the risk management process.

Socio-economic considerations cover a wide range of issues and concerns. There are two relevant international documents which address socio-economic considerations in decision-making with regard to potential risks of GMOs to people and the environment. These are: (a) the Cartagena Protocol on Biosafety of the Convention on Biological Diversity; and (b) the Codex Alimentarius (international food code). Article 26 of the Cartagena Protocol on Biosafety, in particular paragraph 1 states that:

1. The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities.

It is clear in Article 26 of the CPB that countries may take into account socio-economic considerations in making decisions with regard to GMOs. Paragraph 1 of Article 26 defines the limits and conditions when applying socio-economic considerations in decision-making on risks posed by GMOs to the environment. The definition implies that not all socio-economic considerations can be included, but only those where GMOs directly impact biodiversity. It also specifies the condition that when countries decide to take into account socio-economic considerations in decisions on GMOs, it must be done in a manner that is

consistent with other international obligations, which includes treaties of the World Trade Organization (WTO).

Codex Alimentarius guidance documents also state that socio-economic considerations may be taken into account in decisions on GMOs. Unlike the CPB, Codex principles are not legally binding to national legislations. However, Codex principles are referred to specifically in the Sanitary and Phytosanitary Agreement (SPS) of the WTO, which is a legally binding international treaty signed by many countries. (For details, please refer to Module E).

Codex principles on risk management particularly relevant to socio-economic considerations include Section 3.16 of Codex Alimentarius for foods derived from modern biotechnology (2003), which states that:

"Risk management measures for foods derived from modern biotechnology should be proportional to the risk, based on the outcome of the risk assessment and, where relevant, taking into account other legitimate factors in accordance with the general decisions of the Codex Alimentarius Commission as well as the Codex Working Principles for Risk Analysis."

Appendix IV of the Codex Working Principles for Risk Analysis on human health (Codex, 2003) and the Criteria for the Consideration of the Other Factors Referred to in the Second Statement of Principles outlines the points and criteria relevant to socio-economic considerations.

These include:

- other legitimate factors relevant for the health protection of consumers and for the promotion of fair practices in food trade based on the following criteria:
  - other factors should not affect the scientific basis of risk analysis
  - other factors which can be accepted on a worldwide basis, or on a regional basis

- specific other factors should be determined on a case by case basis
- other factors should consider the feasibility of risk management options and concerns related to economic interests and trade issues
- other factors should not create unjustified barriers to trade

The risk management process should:

- take into account an assessment of their potential advantages and disadvantages
- consider the economic consequences and feasibility of risk management options, paying particular attention to the circumstances of developing countries

As can be noted in the above, the existing guidance documents treat socio-economic considerations in general terms. To date, there are still no internationally agreed definitions and scopes of socio-economic considerations and methodologies for analysis and incorporating socio-economic considerations into the decision-making process. Even at the national level and for what may be considered a "legitimate factor" like economic risk-benefit analysis, there are no biosafety regulatory systems that have formally included a benefit assessment within their regulatory structure.

CHAPTER 5

THE RISK ANALYSIS PROCESS: RISK COMMUNICATION

Risk communication is "the interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors and risk perceptions among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions" (Codex Alimentarius Commission, 2003).

RISK COMMUNICATION
The interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors and risk perceptions among risk assessors, risk managers, consumers, industry, the academic community and other interested parties.

Risk communication in this sense is also addressed in Article 23 of the Cartagena Protocol on Biosafety on public awareness and public participation which states that:

1. *The Parties shall: (a) Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of living modified organisms in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. In doing so, the Parties shall cooperate, as appropriate, with other States and international bodies; (b) Endeavour to ensure that public awareness and education encompass access to information on living modified organisms identified in accordance with this Protocol that may be imported.*

2. *The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding living modified organisms and shall make the results of such decisions available to the public, while respecting confidential information in accordance with Article 21.*
3. *Each Party shall endeavor to inform its public about the means of public access to the Biosafety Clearing-House.*

There is wide agreement that effective risk communication is essential at all phases of risk assessment and risk management. It is also recognized that risk communication involves not only risk assessors and risk managers, but also other interested parties like government, industry, academia, consumers, public interest groups and individuals concerned with risk.

Risk communication is essential in making decisions (ILGRA, 1999). It enables all interested parties, not only risk assessors and risk managers, to participate in deciding how risks should be managed.

Communication is also a vital part of implementing decisions - whether explaining mandatory regulations, informing and advising people about risks which they can control by themselves, or dissuading people from risky, antisocial behaviour. Therefore, the main goals of risk communication are: (1) to improve knowledge and understanding on all aspects of the risk analysis process by all interested parties concerned with risk; and (2) to promote interactive communication between risk assessors, risk managers and other interested parties concerned with risks in order to achieve the desired outcomes.

Risk does not have to turn into a crisis if it can be identified, planned for, and dealt with effectively. Good communication is the key. Good Risk Communication is the presentation of a *scientific assessment of risk* in such a way that the public can understand the information of the risk without becoming emotionally involved.

Good risk communication must:

- translate the scientific findings and probabilistic risk assessment into understandable terms;
- explain the uncertainty ranges, knowledge gaps, and ongoing research programmes;
- address issues of credibility and trust;
- understand the public's concern with regard to risk issues, and acknowledge their questions and concerns;
- analyze the conditions needed for the public to acquire relevant information, skills, and participatory opportunities.

Good communication with the public can also help responsible agencies to handle risk more effectively:

- **Lead to better decisions about how to handle risks**

  Considering and integrating a wide number of public and stakeholders' opinions may contribute to formulating well-suited and adequate decisions about the management of a certain risk.

- **Preventing crises**

  Early discussions with stakeholders and the public can help to inform responsible authorities of potential areas of public concern early on. This can enable them to take early action to address those concerns, before they turn into crises. It can be particularly valuable where there are public concerns about risks associated with new technologies, such as GMOs. Engaging a wide range of stakeholders and the public in risk decisions can help ensure that decisions take account of a wide range of views and experience. It can also help responsible authorities to spot aspects of a risk that might otherwise have gone unnoticed. This can be particularly important where action taken to tackle a risk could have a knock-on effect on others.

- **Smoother implementation**

  A key feature of risk management, and of policy-making, is the need to deal with

GOOD COMMUNICATION
Good communication with the public will profit the entire risk analysis process in a variety of ways, e.g. result in better decisions, prevent crises, build trust etc.

different and often conflicting perspectives. Engaging stakeholders and the public at an early stage in decisions about risks can help ensure that decisions better reflect public values and can reduce the scope for misunderstanding, disagreement and bitterness later on. This can make it easier to implement measures to address risks, particularly where these require the public to take action.

» **Empowering and reassuring the public**

Providing clear and accurate information about the nature of risks can help people to make realistic assessments of the risks they face and, where appropriate, to make informed judgments on how to handle risks by themselves. This can in turn help to foster a climate of greater empowerment and reassurance, and reduce the risk of scares.

» **Building trust**

Over time, communication with stakeholders can help to reduce suspicion, and build trust in the information government provides. Open communication can help by bringing people "inside the tent", and by enabling them to see for themselves that decisions have been made on the best available evidence and with the public interest in mind.

Also, effective risk communication can help responsible agencies to:

» explain technical risks more effectively;
» understand the multi-dimensionality of risk;
» anticipate community responses to the intended activities;
» respond to public concerns and misinformation;
» increase the effectiveness of risk management decisions by involving concerned community members;
» improve dialogue and reduce tension between communities and companies;
» build relationships based on trust and respect;
» develop a good reputation with regulators and the public;
» build a foundation for dialogue and shared problem solving before operations begin.

5.1 WHEN TO COMMUNICATE ABOUT RISK

It is widely acknowledged that risk communication is an integral part of the risk analysis process. It is embedded into the risk assessment and risk management processes; two key steps – hazard identification and selection of risk management measures – require effective risk communication to help build trust, reduce conflicts and achieve desired outcomes. In hazard identification, the views and opinions of interested parties about the potential hazards can help define the issues of concern and reduce potential points of conflict. During the selection of risk management options, the risk managers may need to consider factors in addition to the scientific input in the evaluation of a risk. This should involve active participation of stakeholders and other interested parties. Finding a common language that will be clearly understood by all parties is needed in explaining the results and the procedures of the risk assessment and risk management processes.

5.2 APPLYING RISK COMMUNICATION PRINCIPLES IN RISK ANALYSIS

RISK COMMUNICATION PRINCIPLES
Several elements, principles, barriers and strategies for effective risk communication have been identified.

The joint FAO/WHO expert consultation on the application of risk communication to food standards and safety matters identified the elements, principles, barriers and strategies for effective risk communication (FAO, 1999). The principles, applied to risk assessment and risk management processes, are illustrated below:

- *Know the audience*. In the risk analysis process, the different types of audience may include risk assessors, risk managers, government, interest groups and the general public. It is important to listen to and understand their motivations, opinions, concerns and feelings. These are important in the development and delivery of credible information on the risks identified, the decisions made, and the processes used. Understanding the audience's perception of risk can be done through surveys, interviews and focus groups.

- *Involve the scientific experts*. Scientific experts are primarily involved in the risk analysis process in their capacity as risk assessors. They work very closely with the risk managers in arriving at the final decision on any dealing with GMOs. These experts must be able to explain clearly the results of their assessment, including the assumptions and subjective judgments, so that risk managers can clearly and fully understand the risks and consequently formulate their decision.
- *Establish expertise in communication*. The risk analysis process generates enormous amounts of information which is of interest to a wide-ranging audience. Developing credible information and delivering it effectively requires communication expertise. Risk communication experts have to be involved as early as possible. Communication expertise of risk managers and risk assessors has to be improved by training and experience.
- *Be a credible source of information*. In the risk analysis process, the sources of information are risk assessors, risk managers, applicants of the technologies in question, and other interested parties. Information from a credible source will likely be accepted. For example, information from the Codex Alimentarius Commission on food safety assessments will more likely be accepted than information from a company consultant. Consistent messages from multiple sources lend more credibility to the risk assessment. Results of safety assessments by regulatory bodies of many countries on a particular GMO will likely receive higher acceptance. To be credible, the source of information should be perceived as genuinely concerned with the views and opinions on the risk issues, trustworthy, competent, committed and consistent. Timeliness in delivery and up-to-date information to address current issues and problems adds to the credibility of a source.
- *Share responsibility:* There are multiple actors involved in the risk analysis process. These include risk assessors, risk managers, other interested parties and the media. Each has a specific role to play, but have joint responsibility for the outcome. Since science must be the primary basis for decision-making,

all parties involved in the communication process should know the basic principles and data supporting the risk assessment and the policies underlying the resulting risk management decisions.

- *Differentiate between science and value judgment.:* It is essential to separate "facts" from "values" in reporting the results of the risk assessment and decisions made in the risk management process.
- *Assure transparency*. For the public to accept the risk analysis process and its outcomes, the process must be transparent. This means the process and results of risk assessment and risk management must be accessible and available for examination by interested parties, but giving due regard to confidentiality of information (if allowed by regulation).
- *Put the risk in perspective*. In the process of risk analysis, this can be done by emphasizing the information about the risk that is relevant to help the target audience make up its mind. For example, in the decision-making step, the risk manager may examine the risk in the context of the benefits associated with the technology. Risk comparisons that underestimate the concern should be avoided.

5.3 FACILITATING PUBLIC ENGAGEMENT IN THE RISK ANALYSIS PROCESS

Risk communication not only aims at informing and educating the public, i.e. improving the understanding of risk issues, but also at dealing with conflicting views and interests of the regulators, other interested parties and the general public on all aspects of the risk analysis process. Engaging all parties in a responsive and interactive dialogue may not change their individual positions, but may lead to a better understanding of and increased level of acceptance in the decisions made.

FACILITATING PUBLIC ENGAGEMENT
Engaging all parties in a responsive and interactive dialogue may lead to a better understanding and increased level of acceptance in the decisions made.

The need to engage the public in decision-making processes concerning the safety of GMOs to people and the environment is increasingly being recognized. This trend is

clearly presented in the results and background documents of the FAO Biotechnology Forum (Ruane and Sonnino, 2005). The decision-making processes identified where public engagement is needed are risk assessment and risk management, particularly in the approval of GM products. However, there are still no internationally agreed guidelines as to the extent and manner public input can be integrated into the risk analysis process.

The joint FAO/WHO expert consultation on application of risk communication to food standards and safety matters (FAO, 1999) identified steps in the risk analysis process where public input may be considered. The most important is the risk management step, specifically in the identification and weighting of policy and decision alternatives by risk managers. It was suggested that interested parties, whenever practical and reasonable, should be involved in identifying management options, developing criteria for selecting those options and providing input to the implementation and evaluation strategy.

The Institute for Development Studies (IDS, 2003) also considered some of the choices regarding the point at which the public could be involved in the decision-making process in the implementation of regulatory frameworks. In the context of the risk analysis process, some of the choices identified are:
(1) identification of risk issues (what do citizens know, what are they concerned about?); (2) roles, duties and powers of responsible agencies; (3) mechanisms of reporting, public scrutiny and accountability; (4) location and design of biosafety trials. The kinds of processes that then may be used include: (1) engaging with areas of public concern (rather than assuming what people need to know); (2) ensuring openness about applications for biosafety review and commercialization; (3) ensuring openness about the purpose, location and design of biosafety trials; (4) ensuring opportunities for public comment.

BOX 5.1

ENGAGEMENT OF STAKEHOLDERS

Questions that will assist in identifying relevant stakeholder groups

- which branches of government(s) are officially involved in the applicable regulatory process?
- who might be affected by the risk management decision?
- who has information and expertise that might be helpful?
- who has been involved in similar risk situations before?
- who has expressed interest in being involved in similar decisions before?
- who reasonably might be angered if not included?

Example of tactics to engage stakeholders

MEETING TECHNIQUES

- public hearings
- public meetings
- briefings
- question and answer sessions
- focus groups
- workshops
- inclusion of non-scientific stakeholder groups in scientific meetings

NON-MEETING TECHNIQUES

- interviews
- hotlines and toll-free numbers

The kinds of tools which may be considered include stakeholder forums that are accessible and widely advertised and public registers of applications under review, with routine opportunities for public comment and obligations to respond to public comments. Furthermore, it should be noted that the perception of risk is highly subjective and context-dependent. Factors that may influence the public perception of risk, and which therefore need to be considered when engaging in communication with the public and establishing a dialogue on risks and risk analysis processes, include the following:

- **Dread.** Hazards that provoke a risk that is perceived as dreadful tend to evoke stronger fears than something seen as less dreadful.

- **Control.** When an individual feels as though she/he has some control over the process determining the risk faced, that risk usually seems smaller than if it had been decided by a process over which the individual had no control.
- **Natural** or human made. Natural risks (e.g. sun radiation) are usually perceived as less wonying than human-made risks (e.g. anthropogenic sources of radiation) even when facts show that the former present greater risks.
- **Choice.** A risk that an individual chooses usually seems less risky than a risk that is imposed.
- **Children.** Risks to children are generally perceived as worse than the same risk to adults.
- **Awareness.** Greater awareness of a risk increases conscious concern about that risk.
- **Personal exposure.** Any risk seems larger if an individual thinks they or someone they know could be a victim - this helps explain why statistical probability is often irrelevant to people and an ineffective form of risk communication.
- **Risk-benefits trade-off.** When people perceive a benefit from a certain behaviour or choice, the risk associated with it seems smaller (e.g. the benefits of a vaccination are perceived to outweigh the risk of the side effects); if there is no perceived benefit, the risk seems larger.
- **Trust.** Research has shown that the less people trust the institutions that are responsible for exposure to the risk or communication about the risk, the more they will be afraid.

As a final note, IDS emphasized that public participation is highly contextual. While the concerns are similar, there is no “one size fits all” formula for public participation and awareness-raising. What works in some places or in some circumstances will not work everywhere. Appropriate forms of public participation and consultation need to take into account the different situations, sociological differences, capabilities and stages of development of each country.

ANNEX 1

MANAGEMENT OF RISKS IN FACILITIES

A CAUSES OF ACCIDENTS IN LABORATORIES FOR BIOLOGICAL CONTAINMENT

Most **accidents** in containment facilities occur due to inadequately trained staff, poor handling, negligence and lack of adherence to norms of prevention and protection. For such reasons, national and international organizations, such as the WHO, have developed technical guides on general and specific methods that should be taken into consideration in facilities dealing with pathogenic agents.

ACCIDENTS
Most accidents in containment facilities occur due to inadequately trained staff, poor handling, negligence and lack of adherence to norms of prevention and protection.

The probability of accidents occurring when working with pathogens is directly related to the type of work being done, but is generally much lower in facilities in which the personnel are better trained. Training on the following topics should be provided:

- nature of dangerous agents, substances and products that exist in the laboratory;
- work procedures, the means of containment and safety and the means for individual protection;
- use and operation of equipment;
- means for disinfection and sterilization;
- what to do in the case of emergencies.

CAUSES OF ACCIDENTS
Generally, causes of accidents can be grouped into technical factors (equipment etc.) and subjective factors (personnel).

The **causes of accidents** in containment facilities are diverse. Therefore, an assessment of the potential risk has been developed to furnish norms and methods for adequate containment and protection for each type of laboratory and for each situation. To guarantee safety at work with pathogens or GMOs, two important factors should be considered:

- The objective or technical factors regarding the facility and its equipment, in terms of guaranteeing containment and safety.
- The subjective factors, in terms of the people who, in one form or another, are involved in the laboratory processes and who are important in carrying out the work under safe conditions.

Accidents result from circumstances where containment measures and equipment fail or where safety practices and procedures are not followed. Such situations can be caused by personnel obviating inconvenient procedures designed for their own safety and not applying correct containment procedures because of badly maintained equipment and facilities. Independent of their diverse nature, accidents can be grouped according to the factors that cause them:

- Technical factors normally associated with badly functioning equipment, methods and systems of protection, containment and biosafety.
- Subjective factors, related to poor use of equipment and methods, failure to observe technical procedures, poor control over processes, lack of attention, tiredness and other uncontrolled actions.

The causes of contamination can also be grouped by:

- Organizational causes, associated with supervising and overseeing work or the lack of a security procedure.
- Technical causes, associated with methods of protection, equipment functioning, operation of security systems, failure to adhere to GLP and factors concerning safety procedures.
- Human causes, associated with capacity, training and discipline, as well as psychological conditions.

Inherent risk factors associated with security at the facilities and during transport of the biological agents should also be taken into consideration. The possibility of entrance of non-authorized personnel or lax security during transport can jeopardize safety and result in liberation of biological material dangerous to humans, animals and plants.

Training and experience, state of health, prophylaxis and medical monitoring of exposed personnel are important. The general level of training in measures of prevention and protection, and specific work experience with the biological materials being handled, represent main factors in the prevention of accidents. When working at BSL 1, it is sufficient to know the GLP of the laboratory and have general experience in necessary techniques. However, from level 2, and particularly at level 3 and 4, it is necessary to have in-depth training in biosafety and specific experience in working with the samples.

The state of health of the personnel is one of the most important factors to be taken into account in assessing risk. All conditions that might predispose personnel to transmissible infections must be considered, including pregnancy or lactation. In this respect, regular medical monitoring of personnel and adequate prophylaxis must be instituted.

B OTHER RISKS IN FACILITIES: CHEMICAL, PHYSICAL AND PSYCHO-PHYSIOLOGICAL

B.1 Chemical risks

CHEMICAL RISK
Risks posed by the use of chemical agents.

When using chemicals it is important to have accurate information on their properties, so as to be able to identify possible dangers and determine the most appropriate means for their handling.

Internationally established norms and regulations exist on the need to specify the characteristics of a chemical substance on its label.

Classification of chemical compounds

The physico-chemical properties and toxicity of chemical substances in terms of the danger they represent can be classified accordingly:

- toxic and very toxic;
- corrosive;
- irritant;
- inflammable and extremely inflammable;
- combustible;
- noxious.

Chemical substances that cause cellular changes in an organism can be grouped into:

- *Mutagen*: Compounds or substances that produce chemical changes in the composition of the bases of DNA, such as 5-bromouracil, 2-aminopurine, nitric acids and mustard gases.
- *Carcinogen*: Chemical agents whose adverse effects are promotion of tumours in animals and humans. Many of the substances that cause mutations are also carcinogens. Among those used in laboratories are xylol, benzine, benzedine, tar, phenols and sulphur.
- *Teratogen*: Chemicals that produce birth defects following malformation of the foetus.
- *Other:* Among those substances deemed to have a chemical risk there are some that do not represent a high risk, but others that can provoke violent reactions and explode or become extremely toxic. These are termed incompatible chemical compounds and they must be stored and handled with care.

The handling of solvents and gases, as well as ordinary chemicals, is potentially dangerous, but is easily managed with adequate preparation and knowledge. Potential problems arising from mismanagement include electrostatic combustion of organic solvents and the danger of explosion from inflammable gases and peroxides. Many of the chemical substances in current use in facilities can cause dangerous

reactions, such as fires, and have to be stored carefully with full understanding of their properties.

Safety principles and risks associated with chemical substances are summarized in Box B.1:

BOX B.1

SAFETY PRINCIPLES FOR HANDLING CHEMICAL SUBSTANCES

Principles of safety for chemical substances

- Read the labels and other sources of information.
- Pay attention when handling.
- Mind your personal safety by using recommended means of protection.
- Transport the substances in secure containers.
- Do not taste or smell chemicals.
- Minimize vapour production by not leaving containers open.
- Store in ventilated places according to manufacturers' instructions.
- Use ventilated fume cupboards to capture toxic emissions.
- Do not smoke, eat or keep food in laboratories.
- Do not pipette by mouth.
- Recognize symptoms of exposure.
- Inform about all accidents and incidents.
- Do not work wearing contact lenses.
- Know the emergency procedures.
- Know where the emergency equipment is.

Risks linked with chemical substances

- Illnesses and changes in health.
- Fires and explosions.
- Poisoning.
- Contamination of the environment.

General precautions for handling chemical substances

- Use gloves and protective glasses.

- Work in a flow chamber.
- Avoid contact with skin, eyes and mucous membranes.
- Clean splashes immediately with lots of water.
- Do not smoke, eat or drink in laboratories.
- Take note of the symbols for level of danger.

The form (liquid, solid, gaseous) of the chemical substance greatly influences its effect. Short exposure to high concentrations of a substance can result in acute effects, while prolonged exposure to lower concentrations can result in chronic effects, manifested as biological changes that disturb normal functions and impair health and the capacity to work.

A summary of facility design and storage and handling of chemical substances is provided in Box B.2:

BOX B.2

FACILITY DESIGN FOR WORK WITH CHEMICAL SUBSTANCES

General methods the facilities should put in place

- Place two doors in opposition.
- Protect the networks for gas and electricity.
- Ventilation should be sufficient to avoid build-up of vapour, install supplementary ventilation for emergency cases.
- Install emergency high-pressure showers to cope with emergencies.
- Place sinks near to working areas.
- Install an auxiliary storeroom in a well-ventilated place to avoid storage near the areas of work.
- Make sure there are emergency procedures in place.

Storing chemical substances

- Keep inflammable and non-inflammable products separate.
- Maintain products in groups

according to danger posed, corrosives, toxic oxidants etc., making sure that incompatible substances are not brought together.
- Keep substances in their original containers.
- Keep sunlight out.
- Keep heavy containers on the bottom shelves.
- Keep the most reactive substances at the lowest levels.
- Oxidizing agents (ethyl ether, isopropyl ether), once opened, must not be stored for more than six months.
- Carcinogens, inflammables and active poisons require special storage.

B.2 Physical risks

PHYSICAL RISKS
All risks related to physical factors and forces, e.g. mechanical, thermal, electrical, radiation and fire.

Physical risks, posing a considerable danger to personnel, are to be found in all areas of a facility and accidents can be different in nature:

- *Mechanical*: Mechanical accidents most often occur when storerooms are inadequately cleaned, there is inadequate illumination, movement is obstructed and objects are badly located. Motors, centrifuges, compressors and other objects with potential energy, such as gas cylinders etc., represent equipment that needs to be handled with specific attention.
- *Thermal*: Among others, high temperatures can cause burns (ovens, autoclaves) and low temperatures can cause hypothermia (cold rooms, liquid nitrogen).
- *Electrical*: Includes the possibility of shock, fire and the source of ignition for particular reactive chemicals in the laboratory (inflammable vapours and gases). Among the causes are faulty electric cables, bad connections and overloading. In facilities with ovens, incubators, autoclaves etc., there is the risk of electrical discharge and severe burns when handling is incorrect or when precautions are not taken and protective equipment is not used. Such accidents are rare but when they occur can be fatal.

- *Radiation*: Ionizing radiation (alpha, beta, gamma, X-rays, neutrons) is potentially the most serious risk and its sources are radioactive isotopes, X-ray equipment and electron microscopes. Other sources of non-ionizing radiation can be important, such as UV light and lasers. Consequences of exposure to high levels of radiation can be burns to the skin, cancer, alterations to the blood system, reduction in bone marrow, cataracts, immunological defects and death.
- *Fire*: Caused by various sources of heat, faulty electrical equipment, defective electrical wires, incorrect positioning of equipment and handling of inflammable and explosive materials.

It is important that refrigerators are of the domestic type, they should be explosion-proof and it is important that the wiring of the thermostat is outside the refrigerator.

According to WHO, the most common causes of fire in facilities are:

- electrical overload; installing new equipment without considering the consequences of adding equipment to the circuit;
- poor maintenance of the electrical system;
- gas pipes and electric cables that are too long;
- equipment that is plugged in when not necessary;
- naked flames;
- poor handling of phosphorus;
- lack of care in handling inflammable materials;
- explosive and inflammable chemicals stored in regular refrigerators.

Equipment for fighting fires should be situated near to the doors of the facility, in strategic areas in corridors and rooms and should include hoses, buckets and fire extinguishers (including water, carbon dioxide, carbonated ice, foam and bromochlorodifluoromethane (Halon 1211, BCF) extinguishers). Equipment should be regularly maintained and checked.

It is very important to install smoke detectors and alarms as part of a detection system that allows rapid response.

In Table B.2 common types of fire and control methods are given:

Table B.2 | **How to extinguish fires**

Type	Combustible material	Extinguisher				
		Water	Foam	CO_2	Chemical dusts	Special agents
A	Wood, textiles, paper and solids in general	Yes	Yes	Yes[1]	Yes	No
B	Inflammable liquids or solids with low combustion points (petrol, acetone, grease etc.)	No[2]	Yes	Yes	Yes	Yes[1]
C	Electrical equipment	No[3]	No[3]	Yes	Yes	Yes[1]
D	Metals and combustible materials	No[4]	No[4]	No[4]	Yes[1]	Yes

[1] Can be used but less effective
[2] Incompatible with water, with which it can cause fire
[3] Electrical conductor
[4] Violent reactions with water, generating hydrogen and producing explosive mixtures with air

B.3 Psycho-physiological and environmental conditions

An additional risk group is composed of human and environmental factors that can considerably increase the risk associated with other factors. The risks are related to aptitudes and capacities to carry out the work, physical and psychological state of the staff, intellectual capacity, training, working atmosphere and conditions. A large proportion of the problems that can arise during a process with a particular attached risk originate from human error.

Such influencing factors can be:

- physiological state;
- psychological state;
- intellectual capacity and job training;
- conduct;
- psycho-social stress.

Environmental conditions have to be taken into account when carrying out tasks in a range of facilities, including temperature, humidity, ventilation and illumination.

A large percentage of problems that can arise in a process with a determined risk originate from human error, and depend on the level of training, such as in the use of safety equipment. Errors and accidents caused by untrained personnel can ultimately result in serious consequences for personnel and the environment in terms of health damage, pollution and economic losses.

ANNEX 2

PRINCIPLES AND METHODOLOGIES FOR THE ENVIRONMENTAL RISK ASSESSMENT

The following information regarding the establishment of an environmental risk assessment was extracted from legislation of the European Community (EC, 2001). Further guidance literature providing detailed explanations concerning the individual steps of the ERA has been prepared and is available online (EFSA, 2006a,b). Further, the requirements for information that must be submitted when handing in an application for a GMO release prove useful in determining the individual points that must be investigated in the ERA (see EC, 2001, Annex III). Connor *et al*. (2003) have also provided an interesting paper, critically investigating the ERA procedure and general risk perception and discussing major areas of environmental concerns associated with GM crops. The information provided here might serve as guidelines for the establishment of individual country environmental risk assessment procedures and relevant legislation.

A OBJECTIVE

The objective of an ERA is, on a case by case basis, to identify and evaluate potential adverse effects of the GMO, either direct or indirect, immediate or delayed, on human health and the environment which the deliberate release or

the placing on the market of GMOs may have. The ERA should be conducted with a view to identifying if there is a need for risk management and, if so, the most appropriate methods to be used.

B GENERAL PRINCIPLES

In accordance with the precautionary principle, the following general principles should be followed when performing the ERA:

- Identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations;
- The ERA should be carried out in a scientifically sound and transparent manner based on available scientific and technical data;
- The ERA should be carried out on a case by case basis, meaning that the required information may vary depending on the type of the GMOs concerned, their intended use and the potential receiving environment, taking into account, i.a., GMOs already in the environment;
- If new information on the GMO and its effects on human health or the environment becomes available, the ERA may need to be readdressed in order to: (I) determine whether the risk has changed; (II) determine whether there is a need for amending the risk management accordingly.

C METHODOLOGY

C.1 Characteristics of GMOs and releases

Depending on the case, the ERA has to take into account the relevant technical and scientific details regarding characteristics of:

- The recipient or parental organism(s);
- The genetic modification(s), be it inclusion or deletion of genetic material, and relevant information on the vector and the donor;

- The GMO;
- The intended release or use including its scale;
- The potential receiving environment; and
- The interaction between these.

Information from releases of similar organisms and organisms with similar traits and their interaction with similar environments can assist the ERA.

C.2 Steps in the ERA

In drawing conclusions for the ERA the following points should be addressed:

1. Identification of characteristics which may cause adverse effects:
Any characteristics of the GMOs linked to the genetic modification that may result in adverse effects on human health or the environment should be identified. A comparison of the characteristics of the GMO(s) with those of the non-modified organism under corresponding conditions of the release or use will assist in identifying the particular potential adverse effects arising from the genetic modification. It is important not to discount any potential adverse effect on the basis that it is unlikely to occur. Potential adverse effects of GMOs will vary from case to case, and may include:

- Disease to humans including allergenic or toxic effects;
- Disease to animals and plants including toxic, and in some case, allergenic effects;
- Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations;
- Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors;
- Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes conferring resistance to antibiotics used in human or veterinary medicine;

» Effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material.

Adverse effects may occur directly or indirectly through mechanisms which may include:

» The spread of the GMO(s) in the environment,
» The transfer of the inserted genetic material to other organisms, or the same organism whether genetically modified or not,
» Phenotypic and genetic instability,
» Interactions with other organisms,
» Changes in management, including, where applicable, in agricultural practices.

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

The magnitude of the consequences of each potential adverse effect should be evaluated. This evaluation should assume that such an adverse effect will occur. The magnitude of the consequences is likely to be influenced by the environment into which the GMO(s) is (are) intended to be released and the manner of the release.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

Major factors in evaluating the likelihood or probability of adverse effects occurring are the characteristics of the environment into which the GMO(s) is intended to be released, and the manner of the release.

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

An estimation should be made as far as possible of the risk to human health or to the environment posed by each characteristic of the GMO identified as having the potential to cause adverse effects. This can be done by combining the likelihood of the adverse effect occurring with the magnitude of the consequences of any such occurrence.

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

The risk assessment may identify risks that require management and how best to manage them, and a risk management strategy should be defined.

6. Determination of the overall risk of the GMO(s)

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

D CONCLUSIONS ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OR THE PLACING ON THE MARKET OF GMOs

On the basis of an ERA carried out in accordance with the principles and methodology outlined in sections B and C, information on the points listed in sections D1 or D2 should be included, as appropriate, in notifications with a view to assisting in drawing conclusions on the potential environmental impact from the release or the placing on the market of GMOs:

D.1 In the case of GMOs other than higher plants

1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).
2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).
3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.
4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).

5. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.
6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).
7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.
8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.

D.2 In the case of genetically modified higher plants:

1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.
2. Any selective advantage or disadvantage conferred to the GMHP.
3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.
4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).
5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms (also taking

into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.

6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).
7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.
8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.

REFERENCES

Australian Government. 2005. The Risk Analysis Framework. Department of Health and Ageing, Office of the Gene Technology Regulator.

BMBL (Biosafety in Microbiological and Biomedical Laboratories) 2007. *5th Edition*. Washington. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention and National Institutes of Health. Available at: http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL_5th_Edition.pdf

CBD (Convention of Biological Diversity). 2000. Cartagena Protocol on Biosafety to the Convention on Biological Diversity: text and annexes. Montreal. Secretariat on the Convention of Biological Diversity. Available at: http://www.cbd.int

Codex Alimentarius Commission. 2003. Report of the Twenty-Sixth Session. Rome. 30 June - 7 July 2003. Appendix IV. Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization, Rome. Available at: http://www.fao.org/docrep/006/y4800e/y4800e0o.htm#bm24

Codex Alimentarius Commission. 2004. Foods Derived from Biotechnology. Joint FAO/WHO Food Standards Programme CODEX Alimentarius Commission. Available at: http://www.fao.org/docrep/007/y5819e/y5819e00.htm

Conner AJ, Glare TR, Nap JP, 2003: The release of genetically modified crops into the environment. Part II: Overview of ecological risk assessment. The Plant Journal 33, 19-46. Available online at http://www.inai.org.ar/ogm/The%20relese.....part%202.pdf

EC (European Commission). Directive 2001/18/EEC. Available at: http://ec.europa.eu/environment/biotechnology/legislation.htm

EC (Council of the European Communities), 2001: 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. *OJ L 106, 17.4.2001, p. 1–39. Available online at* http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:106:0001:0038:EN:PDF

EFSA (European Food Safety Authority), 2006a: Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO). EFSA Journal 99, 1-100. Available online at http://www.efsa.europa.eu/en/scdocs/scdoc/99.htm

EFSA (European Food Safety Authority), 2006b: Guidance document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use by the Scientific Panel on Genetically Modified Organisms (GMO). EFSA Journal 374, 1-115. Available online at http://www.efsa.europa.eu/en/scdocs/scdoc/374.htm

FAO. 1999. *The Application of Risk Communication to Food Standards and Safety Matters.* Report of Joint FAO/WHO Expert Consultation. Rome 2-6 February 1998. Food and Nutrition Paper 70. Available at: ftp://ftp.fao.org/docrep/fao/005/x1271e/x1271e00.pdf

FAO. 2007. FAO Biosecurity Toolkit. Rome. Available at:http://www.fao.org/docrep/010/a1140e/a1140e00.HTM

FAO/WHO. 1995. *Application of risk analysis to food standards issues.* Report of the Joint FAO/WHO Expert Consultation. Geneva, 13-17 March. WHO, Geneva.

Gelvin, S.B. 2000. *Agrobacterium* and plant genes involved in T-DNA transfer and integration. *Annual Review of Plant Physiology and Plant Molecular Biology*. 51: 223-256.

Hayes, K. 2001. *Environmental Risk Assessment for GMs*. CSIRO Marine Division and Biodiversity Sector. Available at: apec.biotec.or.th/pdf/KeithHayes.pdf

IDS (Institute for Development Studies). 2003. Public Participation and the Cartagena Protocol for Biosafety. A review for DfID and UNEF-GEF, Institute for Development Studies (IDS). By D. Glover, J. Keeley, P. Newell, R. McGee. Brighton. Institute for Development Studies.

ILGRA (Interdepartmental Liaison Group on Risk Assessment). 1999. Risk Communication, A Guide to Regulatory Practice. Health and Safety Executive, Risk Assessment Policy Unit. London.

ISO (International Organization for Standardization). ISO/IEC 17025. 2005. General requirements for the competence of testing and calibration laboratories.

Konig, A., Cockburn, A., Crevel, R,W., Debruyne, E., Graftstroem, R., Hammerling, U., Kimberg, I., Knudsen, I., Kuiper, H.A., Peijnenburg, A.A., Penninks, A.H., Poulsen, M., Schauzu, M. & Wal, J.M. 2004. Assessment of the Safety of Foods Derived from genetically Modified (GM) Crops. *Food and Chemical Toxicology*. 42: 1047-1088.

Küenzi, M., Archer, L., Assi, F., Brunius. G., Chmiel, A., Collins, C.H., Deak, T., Donikian, R., Financsek, I., Fogarty, L.M., Frommer, W., Hamp, S., Houwink, E.H., Hovland, J., Lagast, H., Mahler, J.L., Sargeant, K., Tuijnenburg Muijs, G., Vranch, S.P., Oostendorp J.G. & Treur, A. 1987. The WP Safety in Biotechnology of the European Federation Biotechnology, Safe Biotechnology - 2. The classification of micro-organisms causing diseases in plants. *Appl. Microbiol. Biotechnol.* 27: 105.

Millstone, E., Brunner, E. & Mayer, S. 1999. Beyond 'substantial equivalence', *Nature*. 401: 525–526

NIH (National Institute of Health). 2002. Guidelines For Research Involving Recombinant DNA Molecules. Available at: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

OECD (Organization for Economic Co-operation and Development). 2009. Consensus Documents for the Work on Harmonisation of Regulatory Oversight in Biotechnology. Available at: http://www.oecd.org/document/51/0,3343, en_2649_34387_1889395_1_1_1_1,00.html

OECD (Organization for Economic Cooperation and Development). Good Laboratory Practices, published by the OECD's Environment Directorate, revised in 1998, document ENV/MC/CHEM(98)17. http://www.oecd.org/document/63/0,3343, en_2649_34381_2346175_1_1_1_1,00.html

Persley, G.J., Giddings, L.V. & Juma, C. 1993. Biosafety, The safe application of biotechnology in agriculture and the environment. ISNAR Research Report 5.

Recuerda, M.A. 2008. Dangerous Interpretations of the Precautionary Principle and the Foundational Values of European Union Food Law: Risk versus Risk. *J. Food L. & Poly.* 4(1).

Ruane, J. & Sonnino, J. 2006. Results from the FAO Biotechnology Forum: Background and Dialogue on Selected Issues. FAO Research and Technology Paper 11. FAO. Rome.

Traynor, P., Adair, D. & Irwin R. 2001. A Practical Guide to Containment Greenhouse Research with Transgenic Plants and Microbes.
Available at: http://www. isb.vt.edu/cfdocs/greenhouse_manual.cfm

Traynor, P.L., Frederick, R.J. & Koch, M. 2002. Biosafety & Risk Assessment in Agricultural Biotechnology: A Workbook for Technical Training. East Lansing. Michigan State University.

UNCED (United Nations Conference on Environment and Development). 1992. Rio Declaration on Environment and Development. Rio de Janeiro.

UNEP (United Nations Environment Programme). 1995. UNEP International Technical Guidelines for Safety in Biotechnology.

WHO (World Health Organization). 2004. Laboratory Biosafety Manual. 3rd Edition. Geneva.

USEFUL READINGS

Andersen, M.C., Megan, E. & Northcott, J. 2004. Risk analysis and management decisions for biological control agents: perspectives from theoretical ecology. Available at: http://www.usda.gov/oce/reports/risk/AndersenBioControlReport.pdf

ASTHO (Association of State and Territorial Health Officials). 2002. Communication In Risk Situations. Responding To The Communication Challenges Posed By Bioterrorism And Emerging Infectious Diseases. Available at:

http://www.astho.org/pubs/astho%20risk%20communication%20e-workbook.Htm

Belgian Biosafety Server. 2006. International classification schemes for micro-organisms based on their biological risks. Available at: http://www.biosafety.be/RA/Class/ClassINT.html

Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. 2005. Advisory Committee on Dangerous Pathogens. Department of Health and Department of Environment, Food and Public Affairs. United Kingdom. Available at: www.hse.gov.uk/aboutus/meetings/committees/acdp/050208/acdp88p6.pdf

Caucheteux, D. & Mathot, P. 2005. Biological Risk Assessment: An Explanation Meant for Safety Advisors in Belgium. *Applied Biosafety*. 10(1): 10-29. Available at: http://www.absa.org/abj/abj/051001caucheteux.pdf

Cellini, F., Chesson, A., Colquhoun, I., Constable, A., Davies, H.V., Engel, K.H., Gatehouse, A.M., Kärenlampi, S., Kok, E.J., Leguay,J-J., Lehesranta, S., Noteborn, H.P., Pedersen, J. & Smith, M.. 2004. Unintended effects and their detection in genetically modified plants. *Food and Chemical Toxicology*. 42: 1089-1125. Available at: http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6P-4C004D33&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=02997d8e5098161ab4f9a0ccf3bb81c9\

Chassy, B.M. 2002. Food Safety Evaluation of Crops Produced through Biotechnology. *Journal of the American College of Nutrition*. 21(3): 166S-173S. Published by the American College of Nutrition.

Chen, M., Zhao, J-Z., Collins, H.L., Earle, E.D., Cao, J. & Shelton, A.M. 2008. A Critical Assessment of the Effects of Bt Transgenic Plants on Parasitoids. *PLoS ONE* 3(5) e2284. Available at: http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0002284

Consulting and Audit Canada for Emergency Preparedness Canada. 1995. Managing Biological Risk with an emphasis on risks associated with biotechnology. Available at: http://www.dsp-psd.pwgsc.gc.ca/Collection/D82-54-1995E.pdf

EC (European Commission). Guidance Document for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. 2003. Prepared for the Scientific Steering Committee by the Joint Working Group on Novel Foods and GMOs Composed of members of the Scientific Committee on Plants, Food and Animal Nutrition. Available at: http://www.ec.europa.eu/food/fs/sc/ssc/out327_en.pdf

Falck-Zepeda, J. 2008. Socio-economic Considerations, the Cartagena Protocol for Biosafety and WTO: What are the issues and what is at stake for developing countries of Article 26.1? Paper presented at 12th ICARB Conference: The Future of Agricultural Biotechnology: Creative Destruction, Adoption or Irrelevance. Ravello, Italy. 12-14 June 2008. Available at: http://www.economia.uniroma2.it/icabr/Public//File/Zepeda_Ravello_14_giugno(1).pdf

Fischhoff, B. 1995. Risk Perception and Communication Unplugged: Twenty Years of Process. Society for Risk Analysis. *Risk Analysis*. 15(2). Society for Risk Analysis. Available at: http://www.sds.hss.cmu.edu/media/pdfs/fischhoff/FischhoffUnplugged.pd

Fox, A. 2003. GLP regulations vs. ISO 17025 requirements: how do they differ? *Accred Qual Assur*. 8: 303.

Gupta, K., Karihaloo, J.L. & Khetarpal, R.K. 2008. Biosafety Regulations of Asia-Pacific Countries. Asia-Pacific Association of Agricultural Research Institutions Bangkok; Asia-Pacific Consortium on Agricultural Biotechnology, New Delhi, and FAO (Food and Agriculture Organization of the United Nations), Rome. P.108 + i-x. Available at: www.apcoab.org/documents/bs_pub.pdf

Halsey, M.E. 2006. I*ntegrated Confinement System for Genetically Engineered Plants. Program for Biosafety Systems*. St. Louis, Missouri, USA. Donald Danforth Plant Science Center. Available at: http://www.ifpri.org/pbs/pdf/pbsbriefhalsey.pdf

Hancock, J. F. 2003. A Framework for Assessing the Risk of Transgenic Crops. *BioScience*. 53(.5).

Jaffe, G. 2008. Establishing National Biosafety Regulatory Systems. Key Outstanding Issues Under the Cartagena Protocol for Biosafety. Program for Biosafety System, Brief No. 12. Available at: www.ifpri.org/pbs/pdf/pbsbrief12.pdf

Lehrer, S.B. Potential Health Risks of Genetically Modified Organisms: How Can Allergens be Assessed and Minimized? Agricultural Biotechnology and the Poor. Available at: http://www.cgiar.org/biotech/rep0100/Lehrer.pdf

Lemaux, P.G. 2008. Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part I). *Annu. Rev. Plant Biol.* 59: 771-812. Available at: http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.arplant.58.032806.103840

Meek, S. & Keese, P. 2006. Putting Theory Into Practice – Applying Risk Analysis to the Regulation of GMOs. Melbourne, Australia. Available at: www.gmo-safety.eu/pdf/biosafenet/Meek.pdf

National Research Council. Improving Risk Communication: Free Executive Summary. Committee on Risk Perception and Communication. Available at: http://www.nap.edu/nap-cgi/execsumm.cgi?record_id=1189

Nuffield Council on Bioethics. 2004. The Use of Genetically Modified Crops in Developing Countries: A Follow-up Discussion Paper. London.

OECD (Organization for Economic Cooperation and Development). An Overview of the Workshop: Beyond the Blue Book – Framework for Risk/Safety Assessment of Transgenic Plants. Available at: http://www.oecd.org/dataoecd/7/55/39018232.pdf

OECD (Organization for Economic Cooperation and Development.). 2001. Communicate Risk. Meeting of Senior Officials from Centers of Government on Risk Management, Reykjavik, 22-23 October 2001. OECD.

Peterson, G., Cunningham, S., Deutsch, L., Erickson, J., Quinlan, A., Raez-Luna, E., Troell, R., Woodbury, P. & Zen, S. 2000. The Risks and Benefits of Genetically Modified Crops: A Multidisciplinary Perspective. *Conservation Ecology*. Available at: http://www.consecol.org/vol4/iss1/art13/

Rationalizing and Harmonizing Plant Biotechnology Regulations in Southeast Asia: A Learning Forum. 4-7 March 2008, Miracle Grand Convention Hotel, Bangkok, Thailand. *Note: contains several powerpoint presentations on status of biosafety regulation of various SEAsian countries, and lectures on environmental risk assessment, plant biotechnology*. Available at: http://www.searca.org/web/training/courses/2008/march_c/index.html

Reeves, C. 2007. Managing outrage and crises: Dealing with Risk by Understanding your Audience. Guelph Food Technology Center News, reprint. Number 49.

Risk Communication Manual. 2005. Capacity Building in Biosafety of GM Crops in Asia. FAO (Food and Agriculture Organization of the United Nations). Bangkok. GCP/RAS/185/JPN. Document No. 1.2005.

Risk Communication Tool. 2003. Electronic Resource and Preservation Network. Available at: http://www.erpanet.org/guidance/docs/ERPANETRiskTool.pdf

Romeis, J., Bartsch, D., Bigler, F., Candolfi, M.P., Gielkens, M.M.C., Hartley, S.E., Hellmich, R.L., Huesing, J.E., Jepson, P.C., Layton, R., Quemada, H., Raybould, A., Rose, R.I., Schiemann, J., Sears, M.K., Shelton, A.M., Sweet, J., Vaituzis, Z. & Wolt., J.D. 2008. Assessment of Risk of Insect-Resistant Transgenic Crops to Non-Target Arthropods. *Nature Biotechnology*. 26(2).

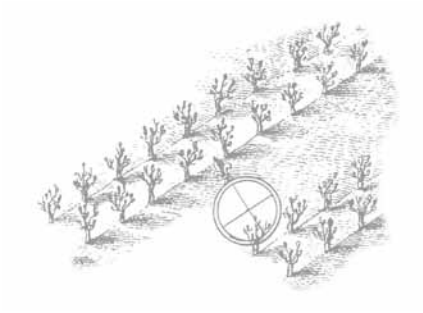
Ruane, J. & Zimmermann, M. 2001. Agricultural Biotechnology for Developing Countries: Results of an Electronic Forum. FAO Research and Technology Paper 8. FAO. Rome.

Spok, A.,, Hofer, H., Lehner, P., Valenta, R., Stim, S., & Gaugitsch, H. 2004. Risk Assessment of GMO Products in the European Union. Toxicity Assessment, Allerginicity Assessment and Substantial Equivalence in Practice and Proposal for Improvement and Standardization. Umweltbandesamt GmBH Wein, Vienna, Austria. Available at: http://www.umweltbundesamt.at/fileadmin/site/publikationen/BE253.pdf

Torgersen, H. 2004. The Real and Perceived Risks of Genetically Modified Organisms. *Europe Molecular Biology Organization Reports*. Special Issue Vol. 5, 2005, pp. 817-821. Available at: http://www.oeaw.ac.at/ita/ebene5/HT_1243.pdf

WB (World Bank). 2003. Biosafety Regulation: A Review of International Approaches. WB Agriculture and Rural Development Department.

Wolt, J.D. & Peterson, R.K.D. 2000. Agricultural Biotechnology and Societal Decision-Making: The Role of Risk Analysis. *AgBioForum*. 3(1): 39-46. Available at: http://www.agbioforum.org/v3n1/v3n1a06-wolt.pdf



Design and layout: Pietro Bartoleschi and Arianna Guida (Studio Bartoleschi)
Cover illustrations elaborated from “l’Encyclopédie Diderot et d’Alembert”
Printed in Italy on ecological paper, Forest Stewardship Council (FSC) certified, May 2011

Biosafety Resource Book

MODULE C

RISK ANALYSIS

provides basic information on biological risks, concepts, principles, and methodologies of risk assessment, risk management and risk communication. It focuses on crop biotechnology and environmental risk assessment of GM crops since these are of immediate interest to most countries.

For additional information please consult
www.fao.org/biotech
or contact
biotech-admin@fao.org

MODULE d

Biosafety Resource Book

TEST AND POST-RELEASE MONITORING OF GENETICALLY MODIFIED ORGANISMS (GMOs)

MODULE d

Biosafety Resource Book

TEST AND POST-RELEASE MONITORING OF GENETICALLY MODIFIED ORGANISMS (GMOs)

Oliver Brandenberg
Alessandra Sensi
Kakoli Ghosh
Andrea Sonnino

Food and Agriculture
Organization of
the United Nations
Rome, 2011

LIST OF CONTRIBUTORS

This module has been prepared based on the lectures and contributions of:

Oliver Brandenberg
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
Institute of Medical Virology,
University of Zürich,
Zürich, Switzerland)

Kakoli Ghosh
Plant Protection and Production Division
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

Leila Macedo Oda
Brazilian Biosafety Association
(Associação Nacional de Biossegurança- ANBio)
Rio de Janiero, Brazil

K. Vinod Prabhu
Division of Genetics
Indian Agricultural Research Institute
New Delhi, India

Orfelina Rodriguez
Centro Nacional de Seguridad Biológica
La Habana, Cuba

Alessandra Sensi
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
EuropeAid Co-operation Office, Unit A.3
Centralised Operations for Europe,
the Mediterranean and the Middle East
Brussels, Belgium)

Andrea Sonnino
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

CONTENTS

MODULE d

LIST OF ABBREVIATIONS

BLP	biosafety level for plants
Bt	*Bacillus thuringiensis*
CBD	Convention on Biological Diversity
CPB	Cartagena Protocol on Biosafety
DNA	deoxyribonucleic acid
EU	European Union
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
ERA	environmental risk assessment
GMHP	genetically modified higher plant
GMM	genetically modified micro-organism
GMO	genetically modified organism
GPS	global positioning system
GURT	genetic use restriction technology
HEPA	high efficiency particulate air
mRNA	messenger RNA
NIH	National Institutes of Health
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
pH	logarithmic measure of acidity/alkalinity of a solution
RNA	ribonucleic acid
SOP	standard operating procedures
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

From the initial research and development of a genetically modified organism (GMO) to its commercial release and placing on the market three different stages, each with specific **biosafety requirements**, can be defined and need to be passed. Namely, these include use of the GMO under containment, confined and limited field trials, and post-release monitoring of the GMO. The specific objectives, procedures and requirements of each of these three areas will be described in detail in this module.

GMOs are not static entities, but are living organisms and as such show all attributes of life: they interact with their environment in a variety of ways, they might show unanticipated effects, they are subject to evolutionary processes, and they follow ecological and biological rules in the same way as every other living organism. The behaviour and attributes of a GMO as well as its interaction with the environment must therefore be considered as dynamic and subject to change over time. This requires careful assessment and evaluation of the potential risks posed by the release of a GMO.

BIOSAFETY REQUIREMENTS
Specific biosafety requirements exist for each stage of a GMO operation; biosafety can be defined as "the avoidance of risk to human health and safety, and the conservation of the environment, as a result of the use for research and commerce of infectious or genetically modified organisms." (FAO, 2001).

Spanning the entire process from the initial research and development of a GMO to its commercial release and placing on the market, a huge amount of information on the GMO needs to be gathered and evaluated. Detailed information is required in order to assess and predict the (agricultural) performance and benefits of the GMO and, most importantly, the risks it poses to human health and environment. A list of recommendations concerning information that should be collected prior to the commercial release of a GMO is provided in Annex 11.

This extensive evaluation and assessment procedure is a bottom-up, iterative process:

- At early research and development stages, no evidence regarding the behaviour and performance of the engineered GMO is available. However, it might be possible to predict to a certain extent such information, including on potential risks, based on the characteristics of the non-modified, recipient organism and the traits encoded by the inserted transgene(s). Once the GMO has been obtained, it can be subjected to laboratory tests to gain information on its characteristics and behaviour under controlled conditions. All research, development and laboratory or greenhouse testing procedures are performed under *Containment*. Containment means that all contact of genetically modified material or organisms with the external environment is prevented, to the extent required by the risks posed by that material or organism. This is usually achieved by a combination of physical and biological barriers.
- If the performance of the GMO under containment is promising and the potential risks it poses are found to be manageable, the testing can proceed to *confined field trials*. Here, the GMO is tested in the open environment, preferably under conditions that resemble its future area of use. However, stringent measures are put in place to confine the release, i.e. to prevent any escape of the GMO or the transgene into the environment and to prevent genetically modified (GM) material from entering human or animal food supplies. Confined field trials are repeated at different scales until all the needed information is acquired.

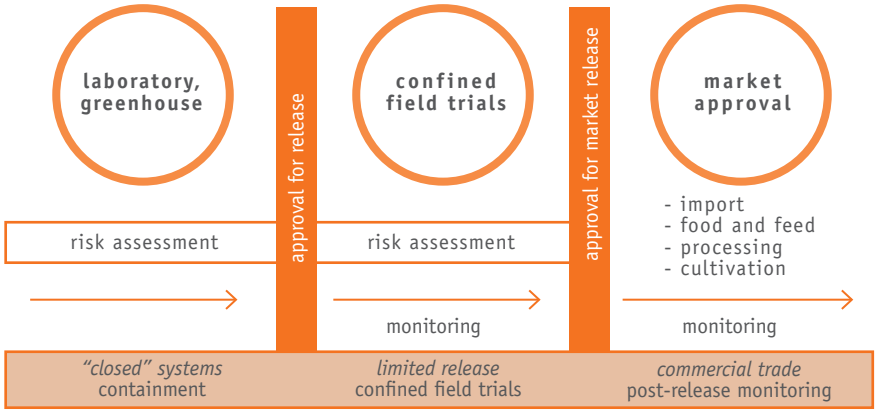
» Once a GMO has passed all testing stages, the risk analysis has been performed with a positive outcome and the approval from the responsible national or international authority has been granted, it may be placed upon the market and released into the environment. From this point on, no measures are put in place that limit the contact between the GMO and the receiving environment, even if specific risk management measures can be requested by the national biosafety authorities. However, it is important to implement *post-release monitoring* procedures to monitor the risks identified in the risk assessment of the GMO, recognize possible new, unanticipated risks and adverse effects, and to quantify the performance and benefits of the GMO. The overall goal of a monitoring programme should be the protection of the productivity and ecological integrity of farming systems, the general environment and human and animal health.

It should be noted that the objectives and procedures as well as the requirements (in terms of financial and organizational inputs, human capacity, infrastructure and equipment) of the three stages can be very different. As mentioned above, the evaluation of a GMO is a **bottom-up, iterative process**: each stage builds upon the information obtained in the previous stages, and possibly provides information that feeds back into these previous stages (Figure 1.1). The ultimate goals of the entire process are to reduce potential risks and prevent potential adverse effects of a GMO on human health and the environment to the maximum extent possible while the risks are not fully understood, to assess and evaluate the risks once they have been identified, and to monitor the manifestation of those risks and potential adverse effects as well as the occurrence of novel, previously unidentified risks once the GMO is released. The objectives, procedures and requirements of each stage are presented in detail in the following chapters. In addition, two small chapters introduce concepts and procedures for GMO traceability, labelling, import and transboundary movements. Thus, all major aspects of GMO deployment, from research and development to market release and international trade, are covered and introduced within this module.

BOTTOM-UP, ITERATIVE PROCESS
The evaluation of a GMO can be described as a bottom-up, iterative process: each evaluation stage during the development, testing and commercial release of a GMO builds upon information obtained during the previous stages, and generates information that feeds back into these previous stages.

Figure 1.1 | **The relation between containment, confined field trials and post-release monitoring of GMOs**

This module will focus on the technical aspects of these processes; for a detailed introduction to the legal background and extensive international frameworks that regulate these processes please refer to Module E: Legal Aspects.



Adapted from: Züghart *et al.*, 2008.

CHAPTER 2

TESTING OF GMOs UNDER CONTAINMENT

Containment, or **contained use**, refers to measures and protocols applied to reduce contact of GMOs or pathogens with the external environment in order to limit their possible negative consequences on human health and the environment (FAO, 2001). Containment measures have to be adjusted to the highest level of risk associated with the experiment, especially when the risk category of the material being worked with is not certain. The risk associated with each GMO should be assessed on a case-by-case basis; accordingly, GMOs are classified into four different risk groups in relation to the risks they pose (see below).

Containment can be achieved by a combination of physical containment structures and safe work procedures (also referred to as good laboratory practices). As an additional feature, biological containment can be included, i.e. "built-in" features of the organism being worked with that prevent its spread, survival or reproduction in the external environment (see Box 2.2). Appropriate containment measures should be applied at each stage of an experiment involving GMOs to avoid release into the external environment and prevent harmful events. This overall objective of a containment system is always the same, however the actual measures that are required can differ, depending on the organisms being worked with (micro-organisms, plants, animals), the scale of the application (large-scale versus small-scale), the research setting (laboratory, greenhouse) and of course the risk classification of the GMOs.

CONTAINED USE
Contained use means any activity in which organisms are genetically modified or in which such GMOs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with the general population and the environment (EU, 1998).

CONTAINMENT FACILITY
The containment facility is the primary structure that ensures containment, by providing physical barriers that limit dissemination of GMO material into the environment into the extent required by the risk posed by the material.

The basic structure of a **containment facility** must meet minimum standards appropriate for the category of risk of the work being conducted. Establishment of the basic minimum structure, adherence to general safety requirements and adoption of good laboratory practices specified for a certain risk group enable any work identified as part of that risk group to be performed within that facility. Therefore, the first step in any operation dealing with GMOs is to classify the GMO and the associated work procedures into one of the four risk groups. Subsequently, one can easily identify the required minimum facility features and good laboratory practices associated with that risk group, and check if the facility that is designated to be used and the standard operating procedures (SOP) for the personnel that are in place comply with these requirements.

2.1 RISK CLASSIFICATION

RISK CLASSIFICATION
A risk classification is the first step that should be performed prior to any GMO operation under containment: The GMO should be classified into one of four risk classes, which dictate the required containment level.

The most common risk classification system is based on four different risk groups, associated with four different biosafety levels (WHO, 2004; NIH, 2009; please refer to Module C: Risk Analysis for a detailed introduction to the topic). Risk groups 1 to 4 represent increasing risk to human health and the environment, similarly biosafety levels 1 to 4 represent increasing strength in the containment measures required to prevent dissemination and spread of the organisms being worked with.

To establish the classification of a GMO, a comprehensive risk assessment should be performed on a case-by-case basis. An initial assessment can be made by classifying an organism according to the following criteria (NIH, 2009):

- Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans.
- Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.
- Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

» Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Subsequently, a comprehensive **risk assessment** should take a detailed look at the organism and the type of genetic manipulation that it is subjected to; factors to be taken into consideration include virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, laboratory operations, quantity being worked with, availability of vaccine or treatment and gene product effects such as toxicity, physiological activity, and allergenicity (NIH, 2009). Such considerations should result in a classification of the organism/project into one of the four risk groups, which also defines the containment level that applies (usually the containment level is the same as the risk group). It should be noted that, to a certain extent, this is a subjective process dependent on the individual researcher/biosafety manager performing the classification.

RISK ASSESSMENT
In order to establish the GMO risk classification a risk assessment needs to be performed, taking into account all relevant characteristics of the organism being worked with and the intended genetic modification(s).

Furthermore, the above-listed criteria are only of limited value when GMOs with a proposed use in agriculture need to be evaluated, because in those cases the potential adverse effects on the environment need to be taken into consideration, in addition to the effects on human health. Detailed lists of factors that need to be evaluated for each organism group (micro-organisms, plants and animals) in order to establish a risk group classification and also define appropriate containment levels can be found in the sections on each organism group below.

2.2 ALTERNATIVE RISK CLASSIFICATION SCHEMES

ALTERNATIVE RISK CLASSIFICATION SCHEMES
Several alternative GMO risk classification schemes exist; however, the four-risk-class system is nowadays widely recognized for classifying GMO operations under containment.

An alternative GMO classification scheme, which is often found in older legislative documents (e.g. EU, 1990) is based on the classification of GMO operations as either type A or type B. Type A is defined as small-scale operations (generally less than 10 litre culture volume) of a non-commercial, non-industrial type, although they can include research and development processes necessary for

BOX 2.1

GENETIC MODIFICATION TECHNIQUES THAT REQUIRE CONTAINMENT

In general, all work that involves recombinant DNA molecules should be performed under containment. For example, the scope of the NIH guidelines is defined as "to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules."

In this sense, recombinant DNA molecules are defined as "(i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above." (NIH, 2009).

Similarly, Council Directive 2001/18/EC (EU, 2001) defines genetic modification, and thus the need for containment measures, as a result of the following techniques:

"(1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;

(2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;

(3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally."

subsequent industrial exploitation. All activities that are not considered to be of type A are automatically classified as type B. This generally implies that the activities take place on an industrial scale and involve production processes and large volumes of material.

In addition to the classification of operations into types A and B, GMOs can be classified into Groups I and II. Group I GMOs are those that meet the following criteria:

- the donor organisms from which the gene or genes derive (parent) do not cause diseases in humans, animals or plants;
- the nature of the vector used in the transformation process is such that it is unlikely to acquire the capacity to produce disease;
- it is unlikely that the resulting GMO can cause disease or adverse effects on the environment.

All GMOs that do not fall into Group I are automatically included in Group II. Such organisms are intrinsic pathogens or have been modified so that they are potential pathogens of humans, animals or plants. However, it is recommended that the risk classification scheme based on the four risk groups described above, together with the four resulting biosafety levels, should be applied. This system is the internationally recognized and accepted system to classify the risks and containment measures for any operation involving recombinant DNA molecules and GMOs.

2.3 NOTIFICATIONS, RECORDS AND EMERGENCIES

2.3.1 Notifications and records

NOTIFICATIONS AND RECORDS
Any GMO operation under containment should be notified to the relevant national competent authority; detailed records of such operations should be prepared and kept.

Any operation that falls under the categories specified in Box 2.1 should be notified to the competent national authority, if such an authority exists. It is recommended that the person wishing to perform operations involving GMOs under containment submits a notification to the competent authority before undertaking such an operation for the first time. This should allow the competent authority to verify that the proposed facility to carry out the operation is appropriate, i.e. that the relevant containment measures are met. The competent authority should confirm that the containment measures and SOPs proposed for the operation limit the hazard to human health and the environment to the required extent.

Any GMO operation should be well documented and the records need to be kept and made available to the competent authority on request. A time span of ten years of record-keeping after the operation has finished is suggested.

2.3.2 Accidents and emergencies

ACCIDENT
An unintentional release of GMOs which presents an immediate or delayed hazard to human health and the environment.

In the event of an **accident**, defined as an unintentional release of GMOs which presents an immediate or delayed hazard to human health or the environment, during the course of the operation, the responsible person should immediately notify the competent authority and provide information that is required to evaluate the impact of the accident and to adopt appropriate counteractions. The information that should be provided includes (EU, 1990):

- the circumstances of the accident;
- the identity and quantities of the released GMO(s);
- any information required to evaluate the effects of the accident on human health and the environment;
- the emergency measures taken.

Information on the occurrence of an accident and the required countermeasures should also be distributed to the general public. Subsequently, an analysis of the causes of the accident as well as of the effectiveness of countermeasures taken should be performed, in order to avoid similar accidents in the future and improve, if necessary, the available countermeasures.

Emergency plans should be developed prior to starting any operation in order to effectively deal with any possible accident and limit the hazard to human health and the environment to the maximum extent possible. The competent authority should ensure that such emergency plans are prepared prior to the operation, that information on safety measures in case of an accident are supplied to persons likely to be affected by the accident and that such information is publicly available (EU, 1990).

EMERGENCY PLANS
In order to react quickly and effectively in case an accident occurs, emergency plans should be developed prior to any GMO operation under containment.

Specifically, the plan should indicate:

- procedures to control the GMO in case of unexpected spread;
- methods to decontaminate or eliminate the effects of an accident;
- methods for disposal or sanitation of plants, animals, soils, etc. that were exposed during the accident or spread.

2.3.3 Other administrative tasks and procedures

In order to allow quick and reliable analysis of whether or not the required safety standards for the biological agent/GMO in question are being followed and met, a checklist should be developed that includes all necessary protocols, safety procedures and facility design parameters. This checklist, or questionnaire, should be prepared in relation to the prescribed biosafety level of the operation. Careful use of such a checklist by the operating personnel and entry of all relevant information should help to maintain the required containment level, avoid unsafe working procedures and identify safety gaps in the experimental design or the design of the facility.

All stipulated regulations, if they are followed properly, will result in meeting the required containment level. An assessment of the training of workers and managers of the containment facility should also be included.

Furthermore, it is recommended that the risk assessment of the GMO operation be revised and updated on a regular basis or when the initial risk assessment is no longer valid. Reasons for this could include changes in the operation (e.g. the scale, available containment measures, changes in work procedures) or the accumulation of new information concerning the organism being worked with that may have significant impact on the risk assessment. Records of the new risk assessment should be kept and the competent authority should be informed of any changes regarding the risk assessment and the applied containment measures.

TRAINING AND SUPERVISION
To ensure safety of personnel working in a containment facility and prevent accidents, regular training and detailed supervision of personnel should be provided.

Regular **training and supervision** should be provided to all personnel involved in the GMO operation. Personnel should be competent to safely perform all working procedures and special care should be taken to ensure that new personnel are made familiar with all working procedures and use of laboratory equipment prior to commencing any work. Training should specifically focus on areas of potential risk as identified in the risk assessment of the GMO being worked with. In addition, all personnel working within the containment facility should be provided with regular health checks.

2.4 CONTAINMENT OF GENETICALLY MODIFIED MICRO-ORGANISMS (GMMS)

GMMS
Specific requirements exist for the risk assessment and containment measures when work with GM micro-organisms is performed.

For the scope of this document, micro-organisms shall be defined as “any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture“ (EU, 1998). This definition, therefore, includes bacteria, fungi, protozoans, algae and viruses as well as eukaryotic cell cultures, amongst others.

The general containment strategies and procedures described above also refer to micro-organisms. The characteristics of each GMM operation should be evaluated and result in a risk classification, which then dictates the containment measures required to ensure the protection of human health and the environment. In cases of uncertainty regarding the risk classification of a GMM operation higher containment measures, corresponding to a higher risk classification, should be applied.

The procedure for the risk assessment of GMMs is described in detail in Annex 1. The ultimate result of such a classification is the assignment of the operation to one of the four risk groups described below:

Class 1: Activities of no or negligible risk, that is to say activities for which level 1 containment is appropriate to protect human health as well as the environment.

Class 2: Activities of low risk, that is to say activities for which level 2 containment is appropriate to protect human health as well as the environment.

Class 3: Activities of moderate risk, that is to say activities for which level 3 containment is appropriate to protect human health as well as the environment.

Class 4: Activities of high risk, that is to say activities for which level 4 containment is appropriate to protect human health as well as the environment.

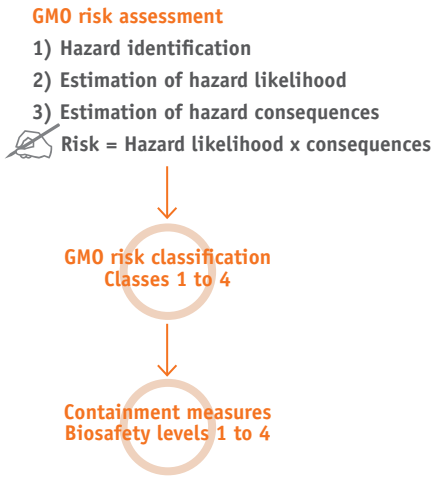
The assessment should also take into account the disposal of waste and effluents, and establish adequate safety measures to control these emissions. The containment levels and physical containment measures (often referred to as biosafety levels), which are appropriate for and correspond to each of the four risk classes described above, are described in detail in Annex 2. In addition to the physical containment measures, principles of good laboratory practice should be put in place and followed by all staff involved with the operation. Guidance for such principles is provided in Annex 3.

Furthermore, considerations concerning the characteristics of the likely receiving environment in case of an accident, the scale of the operation and employment of non-standard operations or equipment may alter the risk class of the operation and similarly affect the containment measures that need to be in place to control that risk level.

It is recommended that the GMM risk assessment and the applied containment level be reviewed on a periodic basis, especially if the containment measures employed are no longer suitable or the risk class of the operation has changed. This may also be the case when new scientific knowledge suggests that the initial risk assessment may be no longer correct.

Figure 2.1 | **The general workflow of the risk assessment, risk classification and adoption of the suitable containment level**

It should be noted that this scheme is not only valid for GMMs, but for every GMO operation that falls under containment requirements (i.e. including genetic modification of plants and animals).



2.5 CONTAINMENT OF GM PLANTS

In this document, plants shall be defined in a broad sense and include higher (vascular) plants, including their reproductive organs such as spores, pollen, seeds, tubers, bulbs, rhizomes, as well as mosses, ferns, algae and aquatic species. In general, the same principles for the risk assessment and containment classification that were laid out in the introduction and for GMMs are also valid for plants. However, the actual risks posed by GM plants and the required containment measures to control and limit these risks and potential hazards are, at least partially, different. The process of risk assessment and the implementation of appropriate containment measures for GM plants are described below.

GM PLANTS
In this document, plants shall be defined in a broad sense and include higher (vascular) plants, including their reproductive organs such as spores, pollen, seeds, tubers, bulbs, rhizomes, as well as mosses, ferns, algae and aquatic species.

2.5.1 Risk assessment for GM plants

In the case of GM plants, the risks posed to the environment are, in most cases, at least equally as important as the risks posed to human health. This is probably because most genetic modifications of plants, especially for envisaged use in agriculture, target growth, survival, herbicide tolerance or pest resistance characteristics, which usually have no implications for human health. Therefore, the risks posed to the environment if an escape of the GM plant were to occur need to be carefully assessed. However, if genetic modifications that target characteristics with possible implications on human health (toxic compounds, allergenic compounds, bioactive compounds in biopharming) are introduced, the risk assessment must pay due attention to these potential hazards.

GM PLANT RISK ASSESSMENT
The general principle for a GM plant risk assessment is identical to other GMO operations; however, for plants the potential adverse effects on the environment are in many cases the primary source of concern, which needs to be taken into account during the risk assessment.

The comprehensive **GM plant risk assessment** should consist of the following steps (Health and Safety Executive, 2007; see also Figure 2.1):

- identification of potential hazards and evaluation of the likelihood that these hazards are realized;
- evaluation of the consequences should these hazards be realized;

- assessment of the risk, i.e. the likelihood of hazard realization and estimated consequences;
- assignment of a risk group and assignment of containment measures appropriate for that risk group.

The detailed procedures and parameters to be taken into account when performing the risk assessment for GM plants are laid down in Annex 4. The ultimate objective of the risk assessment procedure is the assignment of the specific activity with a GM plant to one of four risk classes, and the concomitant definition of containment measures required to control and minimize the risks associated with that risk class.

BIOSAFETY LEVELS FOR PLANTS
Specific biosafety levels for plants, providing detailed information on required containment measures for GM plants, have been defined.

The four risk classes and associated containment measures, also known as **biosafety levels for plants** 1 to 4 (BL1-P to BL4-P) have been defined by NIH (NIH, 2009); brief descriptions of each level are provided below (adapted from Adair and Irwin, 2008). Biosafety levels constitute a combination of facility features and equipment, work practices and procedures, and administrative measures required to maintain a specified level of containment, with the aim of preventing contact between the material being worked with and the outside environment to the appropriate extent. A detailed table summarizing the exact containment measures associated with each biosafety level for plants is provided in Annex 5.

BL1-P: The lowest level of containment is recommended for GM plants for which evidence suggests that they are unable to survive and spread in the environment, and therefore do not pose an environmental risk.

BL2-P: Recommended for GM plants and associated organisms that could be viable in the receiving environment, but are assumed to have a negligible impact or could be easily managed; this includes GM plants with weedy characteristics or capable of interbreeding with related species in the environment.

BL3-P: Recommended for GM plants or associated organisms, including plant pathogens, that have a recognized potential for significant detrimental impact on the environment; this includes genes from exotic infectious agents, gene coding for vertebrate toxins, and plant-associated GM micro-organisms capable of causing environmental harm.

BL4-P: Recommended for readily transmissible exotic infectious agents, possibly in the presence of their arthropod vector, that are serious pathogens of major crops; also included are certain biopharming experiments in which bioactive compounds (e.g. vaccines) are produced in GM plants.

2.5.2 Containment measures for plant research facilities

Research on plants is regularly conducted in **greenhouses** – specialized structures with a transparent or translucent covering enabling the growth of plants inside a controlled environment. Such structures, and the concomitant work procedures, differ significantly from typical laboratory settings and require special considerations regarding containment.

GREENHOUSES
Research and testing of GM plants is regularly performed in greenhouses, specialized structures that allow plants to be grown inside and that require specific containment measures.

The primary objective of plant containment is environmental protection – at least when no risks to human health have been identified. In order to achieve this goal it is recommended to carefully consider all factors that might interfere with containment, including characteristics and behaviour of the organisms being worked with, organism interactions, conduct of experiments, facility (greenhouse) design and limitations, escape routes, and social (personnel-related) factors. A large variety of transport mechanisms for organisms – ranging from micro-organisms to plants – into and out of a containment facility exists, and likewise many opportunities for breaches of containment. These routes include air, water and soil, as well as via personnel (clothing, shoes, etc.), equipment, waste, or via small animal intruders.

Containment measures specifically for greenhouses directed against those factors are briefly described below, while the exact requirements for each plant biosafety level can be found in Annex 5.

- All personnel working in the facility should be familiar with the containment requirements and the work procedures to be followed; SOPs and a reference manual should be established and followed. Problems should be noted and investigated as soon as they become apparent. Routine access should be restricted.
- Care should be taken that dissemination of organisms through clothing, shoes etc. is prevented. Wearing laboratory coats and gloves is recommended even at lower biosafety levels where such measures are not compulsory.
- Physical containment is provided by the facility itself and by equipment employed within that facility; correct handling of the facility and the equipment is required to maintain containment.
- Signs advising of restricted experiments in progress, limited access, potential hazards and contact details of responsible persons should be in place.
- The capability of a greenhouse to isolate organisms from the surrounding environment, as well as to limit entrance of undesired organisms, is strongly affected by the type of glazing, sealing, screening, airflow system, air filtration and air pressure employed.
- Layering of containment measures, i.e. combining several physical measures or combining physical with biological containment measures, can significantly enhance containment (see Box 2.2).
- Special care should be taken when work involves plant-associated micro-organisms, whether or not they are genetically modified themselves. In such cases, the containment measures for micro-organisms should additionally be consulted.
- Storage of material (plant parts, cell culture, seeds) should preferably be performed in lockable repositories.

- Specific requirements exist for safe transfer of material into or out of the facility (use of closed containers, possibly in two layers).
- Prior to disposal, biological material (including soil) must be rendered inactive by validated means (autoclaving recommended).
- Periodic cleaning, as well as disinfection or decontamination of all surfaces or the entire facility should be performed, by means that are efficient for the target organism.
- A pest and undesired organism control programme should be in place; traps or bioindicators can be employed to monitor spread of pollen, insects or viruses etc.
- Alarm systems should be operational to indicate system failures due to technical, human or weather-caused errors and malfunctions.
- Records of experiments should be kept; greenhouses should be inspected periodically.
- Security measures to limit access of unauthorized persons should be in place (fencing, self-locking doors, sensors, security cameras, safety personnel, etc.).
- Researchers should be involved in the planning and design process of a greenhouse facility, since they have the most profound knowledge of the biological aspects of the work to be performed within that facility.
- The site of the facility should be chosen carefully, ideally in an environment that provides the lowest chance of survival and spread of escaped organisms.
- The most suitable greenhouse design offers good security, is long-lasting, easy to clean, withstands repeated disinfection and minimizes hiding places for pests and other organisms.

A detailed description of these points and further helpful information regarding design and maintenance of containment greenhouses are provided by Adair and Irwin, 2008.

BOX 2.2

BIOLOGICAL CONTAINMENT/CONFINEMENT STRATEGIES

BIOLOGICAL CONTAINMENT/ CONFINEMENT STRATEGIES Are highly useful for complementing physical containment measures and thus ensuring effective containment of GMOs.

As pointed out in the text, layering of physical and biological containment measures is considered a most efficient means of achieving containment. Biological containment refers to all measures that directly target the organism being worked on with the aim of preventing sexual or vegetative reproduction and reducing its capability of transgene spread and dissemination, instead of simply providing the physical barriers that contain it in a given area. This can include specific agricultural, horticultural or other work techniques as well as genetic manipulation of the organism to alter its dissemination abilities. These techniques are not only important for research under contained conditions, e.g. in laboratories and greenhouses, but also at later stages of GMO development and commercialization, such as confined field trials or even at the market release stage.

Some of the most common biological containment techniques are listed below.

Horticultural/agricultural management strategies:

- reproductive isolation by removal of flowers prior to anthesis (pollen shed);
- cover flower or seed heads (bagging) prior to pollen or seed release;
- ensure spatial isolation from sexually compatible relatives; specific isolation distances for each crop should be maintained (see Annex 8);
- ensure temporal isolation from sexually compatible relatives, i.e. grow experimental plants in such a way that flowering takes place at different times than that of sexually compatible relatives in the receiving environment;
- stop experiments and destroy plant material prior to flowering;

- if seeds are produced, stringent measures to collect seed, minimize seed dissemination and prevent seed germination in the receiving environment should be in place.

Genetic modification/breeding strategies:

- use male-sterile lines, or sterile triploid lines or interspecific hybrids;
- introduce the transgene into the chloroplast genome; chloroplasts are usually maternally inherited, i.e. no transgene spread via pollen takes place;
- employ cleistogamy, i.e. flowers that do not open, resulting in self-pollination;
- employ genetic use restriction technology (GURT) to yield plants with sterile seeds, or seeds where expression of the engineered trait is repressed (highly controversial due to the implications for farm-saved seeds).

For micro-organisms or insects:

- avoid creating aerosols when working with micro-organisms;
- genetically modify micro-organisms so that survival and replication outside of the experimental setting and/or pathogenicity are compromised;
- when challenging plants with pathogens: use disabled pathogens, provide isolation distances between infected and healthy plants, and eliminate vectors that could transfer the pathogen;
- for insects: use flight-impaired, sterile strains, conduct experiments at time of year or location where survival of escaped organisms is impossible, or choose organisms that have an obligatory relation with the test plant and no other species in the receiving environment.

Further details, including several proposed genetic modification techniques currently at developmental stages, are provided by the Committee on the Biological Confinement of Genetically Engineered Organisms, 2004.

2.6 CONTAINMENT OF GM ANIMALS

GM ANIMALS
As for GM micro-organisms and GM plants, GM animals require specific considerations regarding the risk assessment and the appropriate containment measures.

For the scope of this document, animals shall be defined as all motile, heterotrophic organisms, including vertebrates, invertebrates (e.g. insects) and other multicellular organisms. The first activity the responsible competent authority should perform in the case of GM animals is to check whether the experimenter, institution or organization has the approval of the local animal ethics/welfare committee for dealing with the animal species and the attempted trait modification. If this approval is not granted, the research should be kept in abeyance.

To date, genetic modification of animals has a much lower importance than genetic modification of plants, especially in the field of agriculture: so far, no GM animal with a proposed use in agriculture has been granted approval for market release and commercialization.

The steps towards successful GM animal containment are the same as those outlined above in sections 2.4 and 2.5 on GM micro-organisms and GM plants. First, a risk assessment is performed to evaluate the potential hazards, both to human health and the environment, of the planned GM animal operation. Subsequently, the GM animal operation is classified into one of four risk classes (biosafety levels), each of which requires a specific set of containment measures to minimize the risk of adverse effects on human health and the environment.

Special attention should be paid to the following points:

- potential disturbing effects of GM animals on ecosystems, especially if the GM animal has selective advantages over naturally-occurring relatives;
- invasiveness of non-indigenous GM species that occupy the niche or prey upon indigenous species;
- altered consummation behaviour of GM animals with effects on plant/animal life in the ecosystem;

» expression of biologically active compounds with possible implications for interacting species or human health (biopharming).

Furthermore, the scale and nature of the activity should be considered, e.g. large-scale production of GM animals, or the use of non-standard equipment and facilities such as breeding GM fish in aquaculture facilities (see also Box 2.3).

The exact parameters and procedures for the risk assessment of GM animals are provided in Annex 6. The detailed containment measures for the four GM animal biosafety levels are listed in Annex 7.

BOX 2.3

CONTAINMENT AND CONFINEMENT OF GM ANIMALS: THE CASE OF GM FISH

So far, the containment and confinement measures discussed have mainly focused on GM plants. The simple reason is that the first GM plant was approved for commercial release well over a decade ago and nowadays a wide variety of GM plants are marketed worldwide, with further varieties in development. For GM animals the situation is different: to date, no transgenic animal with agricultural importance has received market approval. However, research in the area of animal transgenesis is active, and one of the fields considered most promising is the creation of transgenic fish, shellfish or crustaceans for use in aquaculture. Obviously, such research and development processes require containment and confinement measures distinct from measures for GM plants or micro-organisms.

Several lines of transgenic fish, covering several species important in common aquaculture, have been created during the last two decades. In most cases, the genetic modification introduced either genes

for growth hormones, resulting in highly accelerated growth rates, or genes conferring increased cold and freeze resistance.

Furthermore, improved disease resistance is also increasingly targeted (Zbikowska, 2003).

Before receiving market approval, the environmental risks of a transgenic fish line need to be carefully assessed. In common aquaculture, fish are often raised in fish cages or similar installations within the open environment with a relatively high risk of escape.

The perceived major risks associated with such an escape of transgenic fish are:

- advantages and higher competitiveness of transgenic fish over wild fish, either of their own or different species, and subsequent displacement of wild fish species and changes in population structures and biodiversity;
- hybridization with wild fish species, resulting in transgene flow to wild species and effects on genetic diversity.

The assessment of these risks is not straightforward, however they need to be evaluated prior to commercial release (see Hu *et al.*, 2007 for examples of risk assessments, mathematical modelling strategies and use of artificial ecosystems for GM fish risk assessment).

In order to limit the risks associated with transgenic fish, containment and confinement measures need to be implemented. Containment measures could include a variety of physical barriers that limit escape of transgenic fish into the open environment in the first place. Ideally, land-based production systems without access to natural waterbodies should be used. In addition to containment structures, biological confinement measures are considered to have a promising role to play in restricting survival, reproduction and transgene flow in cases where GM fish escape from containment.

Bioconfinement strategies include:

- production of sterile fish through induction of triploidy (presence of three chromosome sets per cell) by temperature, chemical or pressure shock of the fertilized egg;
- combining triploidy with all-female (monosex) lines;
- placing the production site in a region where survival of escaped GM fish is restricted, e.g. due to unsuitable water temperature, salinity, pH or other parameters;
- limiting gene flow by placing the production site in a region where no sexually compatible wild species occur;
- several genetic modification strategies aimed at disrupting or limiting reproduction, survival or essential developmental processes should GM fish escape from confinement.

For a detailed discussion of the individual techniques please refer to Committee on the Biological Confinement of Genetically Engineered Organisms, 2004.

All of the listed techniques have specific strengths and limitations, and to date no single technique has been developed that would confer 100 percent protection from any effects of escaped GM fish on wild fish species or transgene flow. Therefore, further research in this area is being performed, and a combination of multiple physical and biological confinement measures is being considered promising to protect from the ecological risks posed by GM fish.

Market approval and commercial release of GM fish will critically depend on a clarification of these issues and the development of appropriate solutions. The commercial release of GM fish, with its anticipated positive effects on aquaculture, should only be performed if the integrity and diversity of aquatic ecosystems can be guaranteed (FAO, 1995).

2.7 GOOD LABORATORY PRACTICE (GLP)

GOOD LABORATORY PRACTICE
A set of standards to describe how research studies should be planned, performed, recorded, archived and reported.

Effective containment and many testing procedures are based on sound laboratory management practices. Many guidance documents refer to these practices in general terms as **good laboratory practice** ("lower case glp") and more specifically as GLP ("upper case GLP"). The former refers to a set of standards used to accredit testing and calibration laboratories (e.g. ISO/IEC 17025, 2005). The latter refers to the OECD Principles of Good Laboratory Practice (OECD, 1998), which sets the standards for specific test studies. Some countries issue their own versions of the GLP Principles based on the OECD Principles of GLP, incorporated as part of national legislations. Please refer to Annex 3 for a summary of GLPs.

The OECD Principles of GLP describe a "quality system concerned with the organizational process and the conditions under which non-clinical studies are planned, performed, recorded, archived and reported" (OECD definition). It is concerned with assurance of data quality (sufficient, rigorous, reproducible) rather than the technical validity of the studies undertaken.

Data generated under GLP are suitable for product registration, mutual acceptance of data among OECD member countries, and to contribute to protection of human health and the environment.

The GLP Principles describe a set of guidelines for the following: test facility organization and personnel, quality assurance programmes, facilities, apparatus, material and reagents, test systems, test and reference items, Standard Operating Procedures (SOPs), performance of the study, reporting of study results, and storage and retention of records and materials.

GLP compliance monitoring is required for mutual acceptance of data. Periodic inspection of test facilities and/or auditing of studies are conducted for the purpose of verifying adherence to GLP principles. Compliance and monitoring are conducted by international, regional or national accreditation bodies, e.g the International Laboratory Accreditation Cooperation (ILAC), Asia Pacific Laboratory Accreditation Cooperation (APLAC) and Australia's National Association of Testing Authorities (NATA). Different countries may require different proofs of compliance with regard to GLP requirements.

CHAPTER 3

CONFINED FIELD TRIALS

CONFINED FIELD TRIAL
After completing the containment stage GMOs can be evaluated in confined field trials. The aim is to evaluate the characteristics of a GMO in the natural environment, while ensuring that dissemination of the GMO or the transgene(s) to the environment is prevented.

As already pointed out in the introduction, the development of a GMO passes through several stages: from initial research and development in the laboratory and subsequent greenhouse testing, both under containment, to confined field trials in the open environment and finally post-release monitoring after the GMO has been placed on the market.

The aim of a **confined field trial** is to evaluate crops with new genetic and phenotypic traits in the natural environment, while ensuring that dissemination of the plant and the transgene is restricted. Field testing is required to collect information on the agronomic performance and the environmental interactions of newly developed crop lines (both from classical breeding and GM crops). This process is essential to establish a detailed environmental risk assessment (ERA) as well as for the characterization and evaluation of the potential agronomic benefits of the new crop line under local environmental conditions. In the case of GMOs, special attention must be paid to ensure environmental protection

and compliance with basic biosafety regulations while performing the trial. This includes detailed requirements for notification and reporting of the trial, a variety of measures to ensure reproductive isolation of the crop, regular monitoring of the trial site and post-harvest land use restrictions, among others. Thus, the planning, conduct and evaluation of confined field trials require a comprehensive, integrated approach including all aspects of the trial. The detailed procedures and confinement measures that are recommended for the successful performance of a confined field trial are discussed below. The discussion will focus on field trials of transgenic crops because they represent the vast majority of GM organisms (see also Box 2.3).

3.1 CHARACTERISTICS OF CONFINED FIELD TRIALS

Confined field trials represent the first introduction of a newly developed GM crop into the environment, being the intermediate step between research and development under containment and unconfined commercial release. They can be defined as "*a small-scale experiment field trial of a genetically engineered plant species performed under terms and conditions that mitigate impacts on the surrounding environment*" (CropLife International, 2005).

CHARACTERISTICS OF CONFINED FIELD TRIALS
Confined field trials usually share several important characteristics, including: a small size, the goal of collecting a variety of data, detailed notification and reporting requirements, strict measures to ensure confinement of the trial, and strict regulations for all processes and personnel involved in the trial.

As such, a confined field trial has several important characteristics:

- It is an experimental activity performed to collect data on the interaction of the GM crop with the local environment and on its agronomic performance, with the aim of formulating recommendations for its potential benefits and establishing a detailed environmental risk assessment.
- It is a small-scale activity, typically around 1 hectare or less.
- The trial is performed with measures in place that restrict the dissemination of the transgene, e.g. via pollen or seeds, into the environment, that prevent the persistence of the plant or its progeny in the environment, and that restrict plant material from entering human or animal food supplies.

- Access to the site is restricted.
- The trial should be notified to the competent authority, regular monitoring of the site should be performed, and reports of the trial should be prepared.
- Trained and informed staff are required for the correct conduct and surveillance of the trial; SOPs and detailed work plans should be established.

Confined field trials are a prerequisite for the unconfined release of GM plants. When a GMO is approved for commercial release, it is assumed that potential hazards for human health and the environment are not significant, as pointed out in the environmental risk assessment. However, for confined field trials the potential hazards may be unknown and are only evaluated throughout the trial, thus stringent measures must be implemented that minimize the exposure of the environment to potential hazards posed by the tested GMO (minimizing risk by minimizing the exposure component).

Confined field trials serve a variety of purposes: First, the agronomic potential of the newly developed GMO and its traits can be tested in the open environment. This should include the investigation of the expression levels of the transgene(s) throughout different plant tissues and different developmental stages, and the effects of the transgene(s) on plant behaviour and characteristics. Second, field trials can be used to produce sufficient plant material for feeding trials and food safety assessments, or for the scale-up of plant material in preparation for commercial release. Finally, confined field trials are required to collect agronomic and environmental data of the GMO that are essential for the completion of the environmental risk assessment. Data to be collected might include possibilities for transgene transfer, impact on target and non-target organisms, evaluation of the environmental fate of the transgene expression products, and any phenotypic or morphological changes of the GM plant that might impact on the environment or agricultural practices.

3.2 RISK MITIGATION GOALS FOR CONFINED FIELD TRIALS

The compliance with biosafety regulations and the safe conduct of confined field trials with GM plants can be achieved by adhering to three risk mitigation processes:

- preventing the dissemination of transgenes into the environment via pollen or seed (reproductive isolation);
- preventing the persistence of the transgenic plant or its progeny in the environment;
- preventing GM plant material from entering human or livestock food supplies.

RISK MITIGATION GOALS
Three primary risk mitigation goals of confined field trials can be defined: preventing the dissemination of the transgene(s), preventing persistence of the GMO, and preventing GMO material from entering food and feed supplies.

Achieving reproductive isolation of the GM plant and thus limiting gene flow via pollen transfer from the confined site to the environment can be achieved by a variety of measures. A number of factors that affect pollen-mediated gene flow via hybridization and introgression to the same or related species need to be considered: the presence of the same or related species in the environment; in case of presence of a related species, whether the two species are sexually compatible, and whether blooming of the two species takes place at the same time; the presence of pollinating vectors; and the fertility and persistence of the progeny plants.

An investigation of these factors requires that the reproduction characteristics of the (unmodified) GM plant are known in detail, such as time of florescence, whether the plant is self or cross-pollinating, pollen dispersal mechanisms and typical pollen travel distances, pollen viability and sexually compatible species. In this respect, it is highly important to assess if the genetic modification has effects on the reproduction characteristics of the plant, compared with its non-modified counterpart. From an assessment of the above-listed factors, appropriate confinement measures for the field trial can be deduced.

Preventing persistence of the GM plant or its offspring in the environment can be achieved by carefully destroying all GM plant material after termination of the trial. A certain period of post-harvest land use restriction should be implemented in order to detect and destroy any volunteer or progeny plants that may come up on the former trial site.

Preventing GM plant material from entering food and feed supplies is a critical point and can be implemented by a combination of measures. These include controlling the transport of GM plant material to and from the trial site, monitoring storage of seed and GM plant material, monitoring the disposal of GM plant material and the disposition of material retained after harvest, and preventing unauthorized harvest from the trial site. The detailed procedures and practices that are required to comply with the risk mitigation measures described above are discussed below.

3.3 PROCEDURES AND PRACTICES FOR SUCCESSFUL CONFINEMENT OF FIELD TRIALS

In this section, the individual procedures and practices that are required to achieve confinement of a field trial are explained in detail. These include prescriptions regarding the conduct of the trial itself as well as regulations with respect to trial planning, trial reporting and notification and post-trial procedures. In general, the first step to be performed to submit an application for the field trial to the relevant competent authority; usually the competent authority specifies the information that needs to be provided in such an application. After the application has been reviewed and the field trial has been approved, the detailed planning and establishment of the field trial may begin.

3.3.1 Transportation and storage of GM material

Successful confinement starts not only at the trial site, but already at the stage of transportation and storage of GM material. It must be ensured that material is handled, packaged, labelled and stored correctly, and that records of all actions are kept.

TRANSPORTATION AND STORAGE OF GM MATERIAL
Confinement starts not only at the trial site: already during transportation and storage of GMO material specific confinement measures should be adopted.

Prior to importing transgenic plant material into a country, relevant import permits need to be obtained from the relevant competent authority (see also Chapter 6 of this module). Adequate records of all transport processes should be prepared and kept; receipts should be issued upon arrival of the GM plant material at its final destination.

GM plant material should be packaged safely for transport and kept separate from other plant material during transportation. Any accidental release of GM material during transport must be avoided. Different recommendations exist for different plant materials: seed should be packaged in three layers, i.e. a primary, secondary and tertiary container, with each layer being independently sealable and capable of preventing release. The primary container should not allow seeds to become trapped within; examples of suitable containers include plastic bags, plastic bottles or metal cans. Suitable secondary and tertiary containers are metal, plastic, cardboard or wooden boxes or crates. For other plant material, e.g. vegetative plant material or material not capable of propagation, two layers of packaging are considered sufficient (Halsey, 2006). After transport, containers should be thoroughly cleaned or may be disposed of by autoclaving, burning or landfill deposition, verifying that all GM plant material has been removed or has been rendered non-viable.

Containers used for transportation of GM plant material must be clearly labelled, allowing quick establishment of the content identity and contact details of responsible persons.

To this end, the label should include:

- the permit number for import or in-country movement (if applicable);
- details of the GM plant material, i.e. plant species;
- form of GM plant material, i.e. seed, whole plants, tubers, bulbs, etc.;
- amount of GM plant material;
- contact details of responsible persons;
- a standard "do not eat" symbol.

Storage of GM plant material should be performed in a way that prevents its release into the environment, and especially its consumption by humans, livestock or other animals. Storage areas should be cleaned prior to and following the storage of GM plant material. Mixing of GM plant material with conventional plant material during storage must be avoided. An inventory of stored material should be prepared and regularly updated, and GM plant material should be clearly labelled. Access to the storage area should be restricted, and signs should indicate the presence of GM plant material.

In the event of an accidental release of GM plant material during transportation or storage, measures should be taken to stabilize the situation and prevent further releases. The site of the accidental release should be marked, and any actions taken to minimize the impact of the release should be documented. The relevant competent authority needs to be informed of the incident immediately.

3.3.2 Establishing and managing the confined trial site

A variety of management procedures should be implemented before, during and following the termination of a field trial in order to ensure the confinement of the trial. Considerations regarding the choice and maintenance of the trial site, requirements for personnel conducting the trial and treatment of equipment are discussed in this section, whereas management measures regarding reproductive

isolation of the GM plant, post-harvest management and monitoring and recording of the trial are discussed in individual sections below. All management measures should be implemented with the aim of achieving the three goals of confinement: preventing transgene spread, preventing persistence of the plant, and preventing plant material from entering food and feed supplies.

The **selection of a trial site** should be based on various considerations. First, the ecosystem in proximity to the trial site should be considered and be taken into account for the environmental safety assessment. This includes the presence of species sexually compatible with the GM plant in the ecosystem adjacent to the proposed trial site. Furthermore, the possibility of maintaining suitable reproductive isolation distances needs to be assessed. Long-term considerations, especially regarding post-harvest land use restrictions, should also be taken into account. Lastly, the presence of neighbouring third parties that might be affected in the event of an accidental release should be taken into consideration.

SELECTION OF A TRIAL SITE
The selection of a suitable trial site is an important step in the planning process of confined field trials and should be based on a variety of considerations (see text).

Following the choice of a trial site, it should be marked and mapped. It is recommended to mark out the four corners of the site, for example with suitable posts, in order to identify it throughout the growing season and subsequent post-harvest land use restriction periods. Global positioning system (GPS) data, if available, might facilitate the recording of the exact trial site. Signs should be put up indicating the presence of GM plants and prohibiting access to non-authorized persons.

A detailed map of the trial site should be established, incorporating the following information:

- contact details of the responsible trial manager;
- identification and/or permit numbers of the trial, if applicable;
- a descriptive land location, i.e. the city, town or region and specifications of how to reach the site from the nearest town;
- exact trial site dimensions;

- total area planted with GM plants, including guard rows;
- distances to permanent markers or surrounding landmarks (telephone poles, fences, roads);
- closest fields of the same species as the GM plant within 1 km distance from the trial site;
- any adjacent natural ecosystems (natural habitats, waterways, forests, etc.);
- the planting date;
- compass directions, with north at the top of the map.

PERSONNEL
It should be ensured that all personnel working on a confined trial site is familiar with the standard operanting procedures and confinement measures that need to be implemented and adhered to.

It should be ensured that all **personnel** working on the trial site during preparation, conduct and post-harvest management of the trial are aware of the material being handled and of the relevant SOPs in place. During the harvest of GM plant seeds or other material, checks should be conducted to ensure that no material is removed from the trial site entrapped in workers' clothing before exiting the trial site. In addition, suitable safety measures should be implemented that limit access to the trial site to authorized personnel, and restrict access of livestock or large animals. Special attention should be paid to restrict consumption of the GM plant material by humans, livestock or other animals.

Before removing equipment from the trial site, it needs to be cleaned of any remaining GM plant material. Methods considered appropriate include manual cleaning, brushing, compressed air, vacuuming or water. It should be verified that the cleaning procedure was successful, i.e. that all plant material has been removed. Additionally, all personnel working within the trial site should routinely check their shoes and clothing for entrapped plant material before exiting the site.

REPRODUCTIVE ISOLATION MEASURES
Measures for reproductive isolation are a core part of confined field trials: a variety of possible measures exist and are selected depending on the specific crop type being tested.

3.3.3 Reproductive isolation measures

Ensuring reproductive isolation by restricting pollen-mediated gene flow from the GM plant being tested to sexually compatible species and thus confining it to

the trial site is a major aspect of confined field trials. Having detailed knowledge of the plant species concerned, especially its reproduction characteristics, is essential for choosing and implementing the most effective measures that will result in successful reproductive isolation of the GM plant. Detailed information on individual crop species can be obtained from background literature, plant researchers, plant breeders or plant and seed producers. Furthermore, the Organisation for Economic Co-Operation and Development (OECD) has developed a series of consensus documents for major crop species, which are available online (OECD, 1997-2009). The different possibilities for ensuring reproductive isolation, which vary according to the crop species concerned, are discussed in the following paragraphs.

Spatial isolation

One of the most widely applied measures for reproductive isolation is to maintain a minimum isolation distance between the GM plant and sexually compatible relatives. The exact minimum distance that should be maintained is dependent on the individual crop species; examples of isolation distances for some of the most important crop species can be found in Annex 8. Sufficient land to establish the required isolation distances needs to be set aside when first planning the field trial. The land within the isolation distance needs to be kept free of the same or related plant species as the GM plant being tested. If such plants are allowed to flower within the isolation distance, a breach of reproductive isolation is supposed to have occurred.

Temporal isolation

Temporal isolation can be employed when the flowering time of a crop species can be predicted with adequate accuracy. This allows the isolation of two sexually compatible crop species, or of a crop species and related wild relatives, by selecting the planting dates so that there is no overlap between their flowering periods. One species must have completed pollen shed completely before or after pollen

shed of the other species, so that there can be no possibilities for pollen-mediated gene flow. Temporal isolation might be difficult to implement due to the inherent variation of ecosystems and living species, resulting in unpredictable changes in flowering times. Temporal isolation therefore needs to be carefully monitored; if two species accidentally flower at the same time, a breach of isolation has occurred.

Removal of flowers

Reproductive isolation of the GM plant being tested can be achieved by identifying and removing all male flowers prior to anthesis. As with temporal isolation, a strict monitoring scheme must be in place in order to identify and remove all inflorescences in time.

Bagging and tenting

Reproductive isolation of the GM plants being tested can also be achieved by limiting pollen-mediated gene flow by physical means. This includes placing bags that prevent pollen release over all inflorescences of trial plants prior to anthesis, or by placing the entire plant within a pollen tent that prevents release of pollen into the environment. In both cases, flowers/plants must remain covered until the pollen has lost its viability.

Early crop destruction

Should flowering of the GM plant being tested not be required for the purpose of the test, early crop destruction can be employed as a means of reproductive isolation. The trial must be terminated and trial plants destroyed prior to anthesis.

Guard rows

The establishment of guard rows, i.e. planting an uninterrupted perimeter border row of conventional plants around the trial plants, is an effective reproductive isolation measure especially for insect-pollinated plants. The guard row acts as a

pollen-trap, thus limiting pollen movement in the environment. Several factors need to be considered when planning guard rows. The required width of the guard row is species-specific, and should be determined on a case-by-case basis.

The conventional plant variety used for the guard rows should flower at the same time as the trial plant, possess similar growth habits and structure, should be planted at comparable densities as the trial plant and should be managed using similar agronomic practices. There must be no gaps present in the guard row which could create problems such as access of equipment to the trial plants. In case the tested GM plant carries traits for herbicide tolerance, care must be taken that the guard row plants are not killed by herbicide application. Strict monitoring should be performed to verify flowering of the guard row and the trial plants at the same time. For post-harvest restrictions and monitoring, the entire area of trial plants and guard rows needs to be included.

Plant modification methods

Instead of providing passive, physical barriers to limit pollen-mediated gene flow, the transgenic plant itself could be modified in such a way that reproductive isolation is ensured. This could include the use of male sterile plants, cleistogamy, or transplastomic plants (integration of the transgene into the chloroplast genome, which, in many plant species, are maternally inherited). Please refer to Box 2.2 for further details on biological containment/confinement strategies.

In case of a **breach of reproductive isolation** through failure of any of the above-mentioned measures, the competent authority needs to be informed and actions taken to limit the release and dissemination of GM plants or transgenes. These could include complete termination of the trial and destruction of any relevant plants within the isolation distance, or simply stricter requirements for post-harvest land use restrictions and monitoring.

BREACH OF REPRODUCTIVE ISOLATION
In case of failure of reproductive isolation measures, a breach of reproductive isolation has occurred which necessitates special procedures, for example immediate termination of the trial or extended post-harvest monitoring.

3.3.4 Harvest and disposal of GM plant material and post-harvest restrictions

HARVEST AND DISPOSAL OF GM PLANT MATERIAL
Harvest and disposal of GM plant material is a critical step, during which it needs to be ensured that no viable material leaves the trial site and that no viable material remains on the trial site.

The termination and harvest of a confined field trial are critical stages that must be carefully monitored, with special attention to two points: preventing GM plant material from persisting at the trial site, and preventing GM plant material from entering food and feed supplies. The following provisions apply both to normal harvest and termination, as well as to early termination and crop destruction, e.g. as a reproductive isolation measure. The competent authority should be informed of the harvest prior to commencing the harvest procedure.

All personnel working on the harvest site should be instructed on the nature of the material being harvested, and a procedure should be implemented to verify that no GM plant material is accidentally released from the trial site entrapped in workers' clothing.

All equipment required to perform the harvest should be cleaned free of plant material both before entering the trial site, and before removing it from the trial site after harvest. Methods considered appropriate include manual cleaning, brushing, compressed air, vacuuming or water. It should be verified that the cleaning procedure was successful, i.e. that all plant material was removed.

All GM plant material should be disposed of directly at the trial site; if transport of GM plant material is required, it should be secured appropriately during transport to prevent any accidental release. All GM plant material that is not retained for research purposes must be rendered non-viable. Recommended techniques to achieve this are heat, incineration, deep burial, chemical treatment, grinding or crushing, or by cultivation into the soil. Following devitalization, GM plant material can be disposed of by incineration, deep burial or cultivation into the soil. If guard rows are used, the guard row plant material should be treated in the same way as the GM plant material.

If any GM plant material from the trial is to be retained for future research purposes, this should be notified to and receive approval from the competent authority.

Post-harvest restrictions and monitoring

Following harvest and termination of the trial, the trial site is subjected to post-harvest restrictions and monitoring; these restrictions begin with the termination of the trial. The aim of these restrictions is to identify and destroy any volunteer plants arising after the termination of the trial, in order to avoid persistence of the GM plant in the environment, prevent gene flow between the GM plant and sexually compatible relatives, and prevent GM plant material from entering food and feed supplies.

POST-HARVEST RESTRICTIONS AND MONITORING
Following termination and harvest of a confined field trial specific requirements exist for post-harvest land use restrictions and monitoring. Time periods for such measures are dependent on the crop type that was tested.

The exact period of post-harvest land use restrictions and the monitoring intervals are dependent on the GM plant species. During this period, all volunteers as well as sexually compatible related species must be identified and removed prior to anthesis. If a breach of this restriction is encountered, the post-harvest restrictions should be extended. Use of the land is restricted to crop species different from the GM crop species that was tested and preferably showing different morphology and growth habits, in order to easily spot and identify any volunteers. Examples of post-harvest periods and monitoring intervals for selected GM crop species are listed in Annex 8.

Regarding personnel, equipment, and measures for devitalization and disposal of plant material, the same provisions as described above for the harvest procedure should apply.

3.3.5 Monitoring, sampling, accidents, reports and records

MONITORING, SAMPLING, ACCIDENTS, REPORTS AND RECORDS
As for GMO operations under containment, confined field trials of GMOs require defined procedures for notification, reporting, accidents and monitoring, amongst others.

Monitoring

Regular monitoring is an integral part of a confined field trial, with the aim of ensuring reproductive isolation, confinement of the trial, and the collection of data on the characteristics and agronomical performance of the GM plant being

tested. It is recommended to monitor the growth and development of the trial plants at least weekly, starting with planting and ending with the termination of the trial, after which specific post-harvest monitoring provisions apply. Monitoring for specific effects, depending on the individual crop and genetic modification involved, should be included in the monitoring plan.

Sampling

Sampling of GM plant material during different stages of the trial might be required in order to investigate the presence of the transgene in different plant tissues and the expression levels of the recombinant protein, or to perform other compositional analyses. The sampling strategy will vary from case to case, but general recommendations for sampling should be followed. These include avoiding cross-contamination between samples, appropriate sample storage in safe containers and at suitable temperatures (usually frozen), and clear labelling of all samples including all relevant information.

Accidents and breaches of confinement

When an accidental release or breach of confinement occurs, certain steps should be taken in order to minimize the impact of such an incident:

- stabilization of the situation, prevention of further releases;
- prevention of consumption of GM plant material;
- recovery of released GM plant material;
- notification to the competent authority;
- marking and recording the exact site of the incident;
- disposal of GM plant material, if required;
- follow-up monitoring and detection.

All procedures and actions taken during an accident should be recorded and documented.

Reports and records

It is recommended that regular reports on the confined field trial be prepared and evaluated by the competent authority. Reports that could be provided include:

- planting report, with details of trial establishment;
- trial progress report(s);
- harvest report;
- incident and corrective action report, if appropriate;
- unanticipated effects report, stating details of such events;
- experimental report, stating all observation and evaluation methods and outcomes of the trial;
- post-harvest report, after the completion of the post-harvest period.

In addition to evaluating the reports, the competent authority could also regularly inspect the field trial site, in order to verify that all relevant measures and procedures to ensure confinement are in place and implemented.

In addition to the reports, it is recommended that records regarding transportation and storage of GM plant material, confinement measures at the trial site, disposal of GM plant material, reproductive isolation measures, planting and harvest procedures, general monitoring, post-harvest monitoring and any accidental releases and the corrective actions taken, be prepared and kept. Records should adhere to certain standards, i.e. be easily readable, include all relevant information (including date and name of the person doing the recording), be prepared promptly after an event, and should be stored in such a way that they are easily traceable and available for review and control.

In Annex 9, a list providing examples of inspection questions that can be used to verify the correct planning, conducting and recording of confined field trials, and compliance with all relevant points listed above, is supplied.

CHAPTER 4

POST-RELEASE MONITORING OF GMOs

COMMERCIAL RELEASE
The ultimate step for a GMO is commercial release. During this stage, no measures are implemented that limit contact of the GMO with the environment, and the GMO is likely to be released on a large scale and in a variety of different environments.

After completing the research and development phases, passing through confined field trials and receiving approval from the competent authority, a GMO can finally be placed on the market and thus be released into the environment. This is a substantially different process compared with confined field trials. First, in confined field trials the risks posed by the GMO are partially unknown, hence measures are implemented to reduce exposure of the environment to the GMO. During **commercial release**, however, the risks are identified and judged to be negligible or manageable, hence no measures are in place to limit exposure of the environment to the GMO. Second, the scale is different: following commercial release a GM plant is free to be grown on very large areas, implying possible scale-related unanticipated effects on the environment. Furthermore, since GMOs are living organisms, they interact with their environment and are subject to ecological laws and processes, possibly resulting in unpredictable effects and behaviour of the GMO following its release.

4.1 CHARACTERISTICS OF POST-RELEASE MONITORING

In order to assess the impact of the identified risks of a GMO on the environment, identify unanticipated effects and evaluate the agronomic performance of the GMO, post-release monitoring is performed. Monitoring can be defined as "*a procedure that involves the systematic measurement of selected variables and processes that may be affected by a given practice*" (FAO, 2005). With respect to GMOs, the aim of post-release monitoring can be described as "*to identify direct, indirect, immediate,*

delayed, or unforeseeable harmful effects that GMO and their application might cause on the environment and human health." (Wilhelm *et al.*, 2003). The results of such monitoring programmes can be used to formulate additional precautions, influence the maintenance, renewal or withdrawal of an approval for a GMO, and can feed back into the risk assessment procedure. GMO monitoring constitutes an early-warning system, since the detection of adverse effects will allow a fast reaction and the implementation of countermeasures at an early stage (Züghart *et al.*, 2008).

POST-RELEASE MONITORING
In order to assess the impact of the identified risks of a GMO on the environment, identify unanticipated effects and evaluate the agronomic performance of the GMO following its commercial release, post-release monitoring is performed.

The release of a GMO could have impacts on the environment at a variety of levels, from single cells to organisms, populations, communities and ecosystems. Due to the variance inherent to all life and ecosystems, effects of GMOs may be difficult to predict in a spatial and temporal manner; they may appear immediately or only after long time spans, and might impact only on the initial site of release or over wide distances and different ecological compartments. Variation will be observed between farming systems, crop types and the environmental contexts. It is therefore recommended to design monitoring plans for GMOs on a case-by-case basis, taking into account all relevant information regarding the individual GMO and the receiving local environment. The choice and establishment of reliable monitoring indicators, which will allow the detection and quantification of adverse effects caused by the release of the GMO and that are based on specific protection targets, is crucial in this respect.

MONITORING PROGRAMMES
To perform post-release monitoring, a monitoring programme should be developed on a case-by-case basis for each GMO release, taking into account the local receiving environment and the characteristics of the released GMO.

The capacity to implement **monitoring programmes** varies from country to country. Developed countries may have the financial and scientific resources to undertake large-scale, long-term post-release monitoring programmes that form a solid basis for decision-making. However, in developing countries the establishment of monitoring programmes represents a greater challenge, due to possible lack of knowledge concerning hazards and risks, limited opportunities for engagement in public debates, less effective enforcement of environmental protection measures and

limited financial, infrastructural or personnel resources for research and development (FAO, 2005). In such cases, a robust monitoring plan based on limited resources should be established that can nevertheless serve the purpose of post-release monitoring as defined above.

As stated, the reasons for monitoring include the verification and reassessment of the findings from the environmental risk assessment, identification of unforeseen effects, the need to meet environmental protection goals and to ensure the productivity and ecological integrity of farming systems.Therefore, the design of the monitoring programme and the evaluation of data are both dependent on and feed back into the environmental risk assessment. Since a basic understanding of the environmental risk assessment is therefore essential to establish and follow monitoring procedures, a brief introduction to this topic is provided in the following section.

4.2 THE ENVIRONMENTAL RISK ASSESSMENT (ERA)

ERA
The objective of the environmental risk assessment is to evaluate, on a case-by-case basis, the impact of a GMO on human health and the environment. Such an assessment is a prerequisite for developing an effective post-release monitoring programme.

The objective of the environmental risk assessment is to evaluate, on a case-by-case basis, the impact of a GMO on human health and the environment. The outcome is a risk classification of the GMO ranging from negligible to high risk, based on a scientific consideration of the potential of the GMO to cause adverse effects and the likelihood that these adverse effects will occur. Direct, indirect, immediate, delayed as well as potential long-term and cumulative effects, caused by the deliberate release of the GMO, should be taken into account (see Box 4.1). The environmental risk assessment is inherently limited in its scope as only identified potential hazards of the GMO can be assessed. Therefore, monitoring serves two purposes: monitoring of the risks associated with a GMO that were identified in the environmental risk assessment (case-specific monitoring), and monitoring for unanticipated effects that were not identified in the environmental risk assessment (general surveillance; see section 4.4 for further explanations).

BOX 4.1

DIRECT, INDIRECT, IMMEDIATE AND DELAYED EFFECTS OF GMOs (EU, 2002A)

Direct effects are primary effects on human health or the environment that are a result of the GMO itself and which do not occur through a causal chain of events.

Indirect effects are effects on human health or the environment which occur through a causal chain of events, through mechanisms such as secondary interactions between organisms and the environment, transfer of genetic material, or changes in use or management practices. Observations of indirect effects are likely to be delayed.

Immediate effects are effects on human health or the environment which are observed during the period of the release of the GMO; immediate effects may be direct or indirect.

Delayed effects are effects on human health or the environment which may not be observed during the period of the release of the GMO, but become apparent as a direct or indirect effect either at a later stage or after termination of the release.

A consistent, science-based procedure and methodology should be followed to establish the environmental risk assessment. The general objectives, principles and methodologies for the environmental risk assessment, as proposed by the European Union, are exemplarily laid down in Module C. These specifications could serve as a template or guidance for the design of individual, case-specific environmental risk assessments. Further information concerning all aspects of risk analysis procedures and principles can be found in Module C: Risk Analysis.

4.3 ESTABLISHING THE MONITORING PLAN

THE MONITORING PLAN
A monitoring plan, developed for a specific GMO release, should include descriptions of the monitoring strategy, the monitoring methodology, and procedures for reporting of the results and relevant triggers for decision-making.

Before commencing any monitoring activity, a detailed monitoring plan should be developed on a case-by-case basis, taking into account the characteristics and intended use of the individual GMO, the environmental risk assessment and the local receiving environment. The available resources and tools to carry out monitoring, in terms of financing, personnel, methodology and infrastructure, should also be taken into consideration when designing the monitoring plan. An analysis of cost-effectiveness should also be included. The monitoring plan should comprise case-specific monitoring, which focuses on the occurrence and impact of potential adverse effects that were identified in the environmental risk assessment, general surveillance, required to identify the occurrence of unanticipated adverse effects, and monitoring for potential cumulative and long-term effects.

Monitoring programmes should be designed so that their purpose and value are ensured: generating information that directly influences effective management and decision-making. In other words, it is important that the information generated by monitoring programmes is received by decision-makers and everybody with a stake in functioning and productive agricultural ecosystems. If this is achieved, correct decisions regarding the preservation of agricultural production systems, ecosystems and rural livelihoods can be reached. The connection between the results of monitoring and decision-making should be clear; monitoring is pointless if the data generated cannot be used. The early integration of all stakeholders and information of the wider public is essential for this process, to ensure that correct decisions are made and implemented at the farm level (Jepson, 2005). It is recommended that the monitoring plan consist of three key sections (Wilhelm, 2003):

- the monitoring strategy, which is based on the objectives and aims to be achieved, the potential effects likely to be observed, and a description of general approaches and timescales to be followed;
- the monitoring methodology, describing all practical aspects of data collection;

BOX 4.2

EVALUATING THE SOCIO-ECONOMIC IMPACTS OF GM CROPS

The assessment of socio-economic effects of GM crops is not a primary goal of the post-release monitoring process. However, such an assessment, as well as the evaluation of the agronomic performance of a GM crop, can be taken into consideration when designing post-release monitoring programmes. In developing countries especially, an evaluation of the socio-economic effects of a GM crop could be important in order to react quickly should adverse socio-economic effects be found to be associated with the introduction of the GM crop. Several methodologies for the assessment of the socio-economic impact are available (Sonnino *et al.*, 2009) and some of them can be adopted in post-release monitoring of GM crops.

In a short paper on this topic (Sahai, 2005) a few points are highlighted that could be taken into consideration when planning socio-economic evaluations in the context of post-release monitoring, using herbicide-tolerant crops as an example:

- In many developing countries, weeding is a major source of rural employment and generation of income. Herbicide tolerance, being a labour-saving strategy, can have negative social and economic implications. In other instances, where the availability of family labour is a limiting factor, herbicide tolerance can have a positive impact.
- Contrary to monocultures in developed countries, weeds in developing countries might not be recognized as such but instead fulfill useful functions. These include use as food and feed and as medicinal plants.
- Possibilities for growing additional crops on field bunds or for mixed farming, both representing an important source for nutrition and income, would be reduced.

Based on such considerations, monitoring indicators to assess socio-economic effects can be developed. The Sustainable Livelihood Approach offers a comprehensive framework for this kind of evaluation.

- data analysis, reporting and evaluation, describing the data evaluation procedure, procedures for reporting to relevant authorities, stakeholders and the public, and providing feedback into the risk assessment and monitoring process.

When designing a monitoring plan the following key steps, established by an expert consultation held at FAO in 2005, could be used as guidance and for identifying the priorities of the process (FAO, 2005). An evaluation of the socio-economic effects of the GMO could also be included in the monitoring programme (see Box 4.2).

- set monitoring programme goals and immediate objectives;
- consult stakeholders, including farmers and managers, regarding the natural resources to develop the goals and immediate objective;
- identify potential barriers;
- prioritize and develop plans to overcome or minimize potential field barriers or otherwise;
- identify potential risks and benefits;
- use stakeholder and expert knowledge of potential risks/concerns and benefits of GM crops, and ways and indicators to measure these factors;
- develop a testing hypothesis to guide actions and decisions;
- ensure that the hypothesis is simple, robust and can be easily tested in the field;
- identify a limited number of potential indicators;
- ensure that the indicators meet the basic requirements of scientific rigour;
- reflect key elements of the hypothesis tested;
- compare with control sites and/or baseline values prior to GM crop release;
- estimate the status and trends in indicator values;
- determine appropriate trigger values for decision-making and action;
- anticipate the range of decisions and actions if triggers are exceeded;
- prepare a follow-up action plan;
- cultivate a transparent and effective process;

- ensure follow-through continued involvement of stakeholder;
- maintain clarity in analysis and reporting, and identify needs; and
- build linkages with policy development and capacity building.

4.4 THE MONITORING STRATEGY

THE MONITORING STRATEGY
The monitoring strategy should be designed in order to allow evaluation of the findings obtained by the environmental risk assessment as well as potential unforeseen effects, taking into account the intended use of the GMO, the scale of the release and the receiving environment.

The monitoring strategy should be designed in order to allow evaluation of the findings obtained by the environmental risk assessment, taking into account the intended use of the GMO, the scale of the release and the receiving environment. Furthermore, the strategy should be able to identify potential effects that were not foreseen in the ERA, or that were associated with a high degree of uncertainty. The strategy should be capable of detecting such adverse effects at an early stage of manifestation to allow fast implementation of countermeasures. All available background information, including information regarding the GMO and the modification event, data from field trials or data from previous releases, should be taken into consideration when designing the monitoring strategy. Importantly, existing monitoring methodologies and observation programmes (e.g. environmental, agricultural or ecological monitoring programmes, food and veterinary surveys, nature conservation or soil observation programmes) should be included in the post-release monitoring strategy to the extent possible and feasible, in coordination with the parties conducting those programmes. The responsibility for the entire monitoring process needs to be clearly assigned, as well as the responsibilities for individual steps of the monitoring process should they be conducted by different parties.

4.4.1 Case-specific monitoring

Case-specific monitoring is performed to investigate the occurrence and significance of any potential adverse effects on human health and the environment associated with the release of a GMO that were identified in the ERA. Specific

CASE-SPECIFIC MONITORING
Case-specific monitoring is performed to investigate the occurrence and significance of any potential adverse effects on human health and the environment associated with the release of a GMO that were identified in the ERA.

hypotheses regarding the occurrence and impact of potential adverse effects should be formulated based on the ERA and tested by scientific means. This should be achieved by systematically recording relevant indicators at representative geographical locations, e.g. spots where exposure of the environment to the GMO is highest or the environment is most likely to be affected. The selection of monitoring indicators, the monitoring methods and the scale (e.g. in terms of number of areas covered) and time frame of monitoring should be determined on a case-by-case basis, taking into account the inherent nature of the GMO and the transgenic event, the receiving environment and the characteristics (e.g. the scale) of the release (EFSA, 2006a,b). For example, if potential adverse effects of an insect pest-resistant GM crop on non-target insect populations have been identified in the ERA, this crop would be the subject of case-specific monitoring using monitoring indicators that describe the impact of that GM crop on the non-target insect species. A clear, testable hypothesis that could be formulated and subsequently tested in this case could be "A change from conventional (insert crop name) to the GM variety will have significant effects on (insert insect name) population density and mortality of insects feeding on the crop". It should be ensured that not only direct and immediate, but also indirect and delayed effects, as identified in the ERA, are included in the monitoring strategy.

In cases where no potential adverse effects are identified in the ERA, no case-specific monitoring is required and monitoring consists of general surveillance and the observation of only cumulative and long-term effects.

4.4.2 General surveillance

General surveillance can be described as routine observation of the geographic regions where a GMO is released; the process aims at identifying the occurrence and impact of unanticipated adverse effects on human health and the environment associated with the release of a GMO that were not predicted in the ERA. As such, general

surveillance should focus on potential indirect, delayed, cumulative and long-term effects, and be performed over extended time periods and multiple geographic locations. As soon as adverse effects are identified, detailed investigations regarding cause and effect chains clarifying the causal connection to the GMO release should be performed (with an hypothesis-based approach as in case-specific monitoring). General surveillance is adequate for monitoring any GMO in any receiving environment since it is not based on an ERA. The drawback is that no hypotheses that can be tested with directed experimental approaches can be formulated, and thus general surveillance is potentially unlimited in its scope. Since no hypotheses can be tested, it is difficult to choose appropriate monitoring indicators that can indicate the occurrence of an adverse effect. Therefore, it is recommended to focus general surveillance on specific environmental protection targets and the occurrence of environmental damage (Bartsch, 2005; see section 4.5.1).

GENERAL SURVEILLANCE
General surveillance can be described as routine observation of the geographic regions where a GMO is released; the process aims at identifying the occurrence and impact of unanticipated adverse effects on human health and the environment associated with the release of a GMO that were not predicted in the ERA.

For general surveillance, an effect can be defined as an alteration in a parameter that lies beyond the normal variation of the agricultural/ecological system. A good starting point for general surveillance would be an investigation of the receiving environment and the exposure level to the released GMO.

Subsequently, it could be determined whether:

- any unanticipated effects are occurring;
- the observed effects are adverse;
- the adverse effects are caused by the release of the GMO.

This evaluation should also include monitoring for potential adverse effects on human health. Obviously, what constitutes an *adverse* effect needs to be defined: for example, the persistence of a GMO in the environment or transgene flow to other species might not be regarded as adverse effects in themselves. However, if such events are associated with, for example, increased weediness or invasiveness, the effect would be defined as adverse (EFSA, 2006a).

4.4.3 The importance of baselines

BASELINES
The baseline status of the receiving environment, i.e. the environment without influences of the GMO in question, is required as a reference point against which all data collected by monitoring can be compared.

The identification and evaluation of effects caused by the release of a GMO through the implementation of a monitoring programme can only be performed if the baseline status of the receiving environment is known. The baseline is required as a reference point against which all data collected by monitoring can be compared. The identification and evaluation of adverse effects are only possible if baseline data for the chosen monitoring indicators that describe the behaviour of these indicators in a GMO-free system state are available. Ideally, the baseline system should differ from the GMO system only in the presence/absence of the GMO.

To obtain such baseline data, two approaches are possible:

- comparison of the system state before the GMO was released with the system state after the GMO release (subsequent comparison);
- simultaneous comparison of an area not exposed to the GMO with an area exposed to the GMO (time-parallel comparison).

Establishing a baseline by subsequent comparison requires monitoring of the system prior to the GMO release; a time frame of three to five years is recommended. However, subsequent comparison is strongly influenced by the variation inherent to natural systems. For example, an insect population (such as the exemplary non-target insect population described in 4.4.1) might show strong variation from one season to another without being reasonably predictable, which would severely limit the suitability of this insect as a GMO monitoring indicator for subsequent comparison. Therefore, time-parallel comparison provides an essential alternative and is especially useful when environments are highly dynamic (EU, 2002b). Ideally, both baseline assessment strategies should be used to complement one another.

The choice of monitoring indicators used to evaluate the state of the receiving environment in a GMO-free condition depends on the suitability of these indicators to assess, subsequently or in parallel, the GMO-related effects on the environment (see section 4.5.1). Using existing environmental observation programmes could provide valuable baseline data, possibly over many years and different sites, concerning the receiving environment prior to any GMO releases.

4.4.4 Time periods for monitoring

TIME PERIODS FOR MONITORING
Time periods for post-release monitoring should be defined in order to detect not only immediate effects but also delayed effects associated with the release of a GMO.

In order to detect not only immediate effects but also delayed effects associated with the release of a GMO, sufficient time periods should be allowed for monitoring. The probability of a specific effect to occur over time, if such a probability can be assigned, should be taken into account. The duration of the release should also be considered; a long release period might favour the establishment of cumulative effects. Furthermore, the duration of monitoring is not necessarily restricted to the duration of the release, but might well extend over the termination of the release. Characteristics of the individual GMO, e.g. its average lifetime, generation time, lifetime of seed banks and risk for persistence in the environment, should serve as guidance for assigning appropriate monitoring periods. The time period should not be fixed, but be adaptable in response to results obtained by the monitoring procedure (EU, 2002b).

4.4.5 Making use of existing monitoring programmes

As stated in previous sections, existing agricultural, environmental, ecological or other related observation or conservation programmes could be integrated in the monitoring plan to obtain data either on the baseline state of a system or on adverse effects caused by the release of a GMO. For example, in cases where routine agricultural evaluations at the farm level are performed, simple surveys

EXISTING MONITORING PROGRAMMES
Existing agricultural, environmental, ecological or other related observation or conservation programmes could be integrated in the monitoring plan to obtain data either on the baseline state of a system or on adverse effects caused by the release of a GMO.

on the observation of adverse effects associated with GMOs (e.g. dissemination, volunteer plants, etc.) could be included (EU, 2002b). Furthermore, collecting information from growers and seed suppliers, e.g. data on GM seed sales, areas sown and crop management techniques (such as obligations to use refugia as an anti-pest resistance strategy, see Box 4.3) could be useful in establishing a monitoring programme.

However, for many existing programmes relevant data for GMO monitoring is unlikely to be obtained, simply because they have been designed for other purposes and thus the targets as well as the methods for data collection and analysis are not suitable. Furthermore, in developing countries in particular the availability of complementary monitoring programmes is likely to be limited (EFSA, 2006a; FAO, 2005).

If existing monitoring programmes are to be integrated into the post-release GMO monitoring plan, the consistency and reliability of data collection and data quality of these programmes should be ensured. Both the questions of which potential adverse effects of the GMO release will be detected by those programmes and which additional measures are required to detect effects that are not covered should be evaluated. Furthermore, should different programmes be used as data sources, methods to collect, analyse and integrate these data need to be developed (EFSA, 2006a).

4.5 THE MONITORING METHODOLOGY

After the monitoring strategy has been defined, concrete procedures and methodologies determining how the monitoring should be performed can be worked out. This includes the choice of monitoring sites, monitoring indicators and procedures for sampling and data collection.

BOX 4.3

DEVELOPMENT OF PEST RESISTANCES AND REFUGIA

One of the major traits targeted by genetic modification of crops is pest and disease resistance. Frequently, resistance against specific insect pests is achieved by expression of the *Bacillus thuringiensis* (Bt) *cry* genes, also known as Bt endotoxins. However, there are concerns that the widespread release and cultivation of GM crops with pest or disease resistance traits poses a high selection pressure on the pest population and leads to development of a pest population that is no longer susceptible to the GM crop resistance mechanism. Development of such an adapted population of the pest species – also referred to as resistance - would lead to failure of the GM crop pest resistance mechanism and thus failure to protect the crop from the pest.

To avoid this, specific crop management techniques can be employed that minimize the development of pest populations that have overcome the crop resistance mechanism. With regard to Bt crops, the most common resistance management strategy is based on the use of GM crops with a high level of Bt gene expression and the concomitant deployment of a refuge consisting of non-GM, pest-susceptible crops (the high dose/refuge strategy). The basis of this strategy is the assumption that the development of insects that are resistant to Bt endotoxins is conferred by recessive mutations which have only low allele frequency within the insect population. Due to the high level of Bt endotoxin expression in the GM crop, only the very rare insects homozygous for the mutant allele will survive on the GM crops. The deployment of a refuge of non-GM crop close to the GM crop area will ensure that the rare mutant homozygous resistant insects surviving from the GM crop area mate with

non-mutant, susceptible insects from the refuge. Therefore, their offspring will be heterozygous for the mutant allele and thus be susceptible to the GM crop.

Depending on the crop and the local conditions, it is recommended that refuges consist of 20 to 50 percent of the area that is planted with GM crop. Mathematical simulations and experience from the field indicate that deployment of this strategy, possibly embedded in an integrated framework of pest management, can delay the development of resistant pests for several decades (Conner *et al.*, 2003; EPA, 2008).

However, especially in the case of small-scale, resource-poor farmers in developing countries, the deployment of refuges might not be economic, or might be neglected due to lack of knowledge (Sahai, 2005). Therefore, it is recommended that compliance with refuge recommendations and evaluations on the development of resistant insect populations be integrated into post-release monitoring programmes. This could help to ensure that refuge recommendations are being followed and that GM crops expressing pest resistance traits maintain their value.

4.5.1 Selecting monitoring indicators

The identification and selection of indicators/parameters to be monitored is a major and decisive step in the entire monitoring process. A major criterion for the selection of indicators is their potential to indicate changes induced by the GMO release. The selection of monitoring indicators should be performed on a case-by-case basis, based on the characteristics of the GMO and the receiving environment. The conclusions of the ERA of a GMO will be helpful in identifying suitable monitoring indicators. For example, if a GM plant expresses Bt proteins

directed against a specific insect pest, that insect species should be monitored to determine the effect of the Bt toxin expression. However, if potential adverse effects resulting from the Bt toxin expression on a non-target insect population have been identified in the ERA, that non-target insect species should also be monitored to assess the occurrence of adverse effects (see also section 4.4.1 on case-specific monitoring).

General considerations for the choice of monitoring indicators include:

- measurability of the indicator, i.e. the possibilities of collecting reliable data concerning the indicator, and adequacy of the data in terms of statistical power;
- availability of and comparability to baseline data;
- relationship and interaction of the indicator with the GMO, either direct or indirect;
- distribution and abundance of the indicator, preferably widespread and high;
- importance of the indicator for ecosystem processes and functions;
- ability of the indicator to represent protectable items.

A list of possible effects of GMOs on human health and the environment, and thus topics for which suitable indicators should be identified, is provided in Table 4.1.

As pointed out in section 4.4.2 on General Surveillance, it may be difficult to identify suitable indicators for monitoring the occurrence of unforeseen and unanticipated adverse effects. This is simply due to the fact that, since the effects are unforeseen, one cannot predict if such effects will occur at all, and if so, which indicators will be suitable to indicate such effects. Therefore, it has been proposed that general surveillance focus on general environmental protection goals and environmental damage (Bartsch, 2005). In this respect, **environmental damage** can be defined as "*a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly*" (EU, 2002b).

MONITORING INDICATORS
The selection of monitoring indicators is crucial for successful post-release monitoring. A major criterion for the selection of indicators is their potential to indicate changes induced by the GMO release. The selection of monitoring indicators should be performed on a case-by-case basis, based on the characteristics of the GMO and the receiving environment.

ENVIRONMENTAL DAMAGE
One possible approach to identify monitoring indicators is to focus on environmental damage, which can be defined as "a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly".

Damage can manifest itself on environmental protection targets, such as protected or endangered species and natural habitats, water and land including associated biodiversity, ecosystem function and human health, including all services and goods associated with these protection targets. It needs to be assessed if a GMO release negatively and significantly influences any such parameters by collecting reliable data and comparing them with the baseline state of the system. However, care must be taken to verify that any effects are indeed caused by the GMO and not just a variation due to natural causes or within the limits of natural fluctuation. Taken together, environmental protection goals could provide a suitable starting point for defining the indicators and monitoring processes for general surveillance.

Table 4.1 | **Potential impacts of GMOs on human health and the environment for which suitable indicators should be identified in order to assess the occurrence of these effects**

Spread and escape of genetically modified plants into the environment
Volunteers in subsequent crops
Hybridization and introgression with wild relatives and feral crop plants, establishment of hybrids
Effects on non-target flora and fauna in cultivated areas and non-target environments
Secondary infestation of crops and hybrids with bacterial, fungal and viral phytopathogens
Consequences of altered farming practice
Effects of herbicide tolerance technique
Development of crop and weed resistance
Effects on phytophagous invertebrates and their antagonists
Effects on interrelations of the food web
Effects on grain- and plant-feeding mammals and birds
Effects on soil functions
Effects on soil fauna and flora
Horizontal gene transfer on micro-organisms
Effects on water bodies and water organisms
Effects on species biodiversity and habitat diversity
Unexpected gene expression
Unexpected physiological and biochemical plant properties
Effects on human health: toxicity, pathogenicity, allergenicity, nutritional quality

Adapted from: Züghart *et al.*, 2008.

4.5.2 Selecting monitoring sites

Careful choice of monitoring sites is crucial for a successful post-release monitoring programme. The number of areas chosen for monitoring should be sufficient to allow sound statistical analysis of the collected data. Choosing and distributing monitoring sites appropriately enables a carefully designed and systematic monitoring system to be representative for large areas.

SELECTING MONITORING SITES
The choice of monitoring sites is the next important step for any monitoring programme. The number of areas chosen for monitoring should be sufficient to allow sound statistical analysis of the collected data and be representative for larger areas where the GMO is released.

Considerations for the selection of monitoring sites include (Züghart *et al.*, 2008):

- representativeness of sites exposed to GMOs, with special focus on sites under repeated or long-term exposure;
- representativeness of ecological regions containing the chosen monitoring indicators;
- availability of sites already under investigation by complementary monitoring programmes;
- sites facilitating spread or persistence of GMOs due to favourable environmental conditions.

Equally important is the choice of appropriate reference/control sites; such sites must meet minimum requirements regarding representativeness of environmental conditions and comparability to the sites exposed to the GMO to allow meaningful statistical analyses and conclusions to be drawn.

When determining the areas to be monitored the characteristics of the individual GMO (such as its preferred ecological niche, reproduction and growth characteristics, etc.), as well as the ecosystems most likely to be affected by its release, should be carefully considered. If potential adverse effects associated with the release of a GMO are identified and specified in the ERA, the choice of monitoring sites will be straightforward because the areas, and possibly even single parameters, most likely to be affected by the GMO are known. If, however, no specific adverse effects

are identified and general surveillance without concrete testing of hypotheses is performed, the choice of monitoring sites is more difficult. In such cases, the inherent characteristics of the GMO and the occurrence of the selected monitoring indicators at a given site are prime considerations for the determination of suitable monitoring sites. Examples of relevant sites include the fields where a GM crop is grown and the surrounding habitats, i.e. sites that receive the highest exposure to the GMO.

4.5.3 Sampling and data collection methods

SAMPLING AND DATA COLLECTION
The choice of sampling and data collection methods depends on the selected monitoring indicators and monitoring sites. The methodologies used should be scientifically sound and appropriate for the experimental conditions; critical considerations include reproducibility, detection limits, availability of appropriate controls, and specificity and selectivity of each method.

The choice of sampling and data collection methods depends on the selected monitoring indicators and monitoring sites. The methodologies used should be scientifically sound and appropriate for the experimental conditions; critical considerations include reproducibility, detection limits, availability of appropriate controls, and specificity and selectivity of each method. The required sample sizes and sampling frequency required to produce statistically valid results should be defined by statistical means.

Sampling should take into consideration the time and space when potential adverse effects associated with a GMO release are likely to be highest. For example, if a GM crop targets a specific insect pest, sampling should be performed at times when exposure to that insect population is highest. Equally, if a transgenic protein is only expressed in the roots and no other plant parts, sampling should be more focused on soil effects of the GM plant (Layton, 2005). Of course, this does not mean that manifestations of adverse effects at other temporal or spatial points should be neglected.

It is likely that no validated standard methods are available for investigating each monitoring indicator. In such cases, one should adapt available methods to the extent possible and build on the experience of previously performed monitoring

BOX 4.4

MONITORING GM MICRO-ORGANISMS (GMMS)

Genetic modification of micro-organisms is considered to have a promising role to play in obtaining micro-organisms with anticipated usage for bioremediation, protection of plants against pests and diseases or enhancement of symbiosis between plants and beneficial micro-organisms, amongst others. The impact of a GMM release on human health and the environment needs to be carefully assessed and monitored, as for every other GM organism.

However, monitoring GMMs in the environment presents particular difficulties and challenges. In contrast to most GM animals and plants, no direct visual detection of GMMs is possible due to their small size. This requires detection and quantification of GMMs in the environment, and assessment of their potential effects, by laboratory methods. Suitable methods include microscopy, detection of modified DNA via PCR, microarrays and selective plate counting, amongst others (see Module A: Agricultural Biotechnology and Jansson *et al.*, 2000 for detailed introductions to GMO detection and quantification techniques).

Furthermore, specific requirements exist for sampling and statistical analyses. Small amounts of soil may contain billions of bacteria and other micro-organisms representing thousands of different species. This challenges the sensitivity of many available methods for detecting a specific micro-organism, possibly present in only low numbers within the sample. The statistical problems associated with sampling and detection limits are discussed by Heinemann and Traavik (2004), using horizontal gene transfer between GM plants and soil micro-organisms as an example. Further improvement in this area is needed to fully assess the impacts and behaviour of GMMs in the natural environment. An example of a long-term field trial of GMMs is provided by Corich *et al.*, 2007.

programmes as far as possible. Standard ecological sampling and data collection methods should be available and include traps, visual observation and specific sampling techniques e.g. for soil or plant material, possibly in collaboration with subsequent laboratory analyses. Parameters that can be investigated using such techniques include species number, growth rates, biomass, reproduction rates, population increases/decreases and genetic diversity (EU, 2002b). Surveys are an alternative means of data collection, e.g. standardized surveys distributed to farmers that allow the declaration of GMO-related effects and procedures, such as the occurrence of volunteers and persistence of the GMO or changed farming and crop management techniques. In general, it should be specified how, by whom and how often data are collected and collated. The availability of trained personnel to perform sampling and data collection is critical for the entire process.

4.6 DATA ANALYSIS, REPORTING AND REVIEW

DATA EVALUATION
Data analysis should be performed with validated statistical procedures, verifying also the quality of the obtained data. The results of such statistical analyses should allow subsequent decisions to be formulated on a sound scientific basis.

Following the sampling and data collection, the collected data need to be analysed, reported to relevant decision-makers and the public, and fed back into the risk assessment procedure and the design of the monitoring plan.

4.6.1 Data evaluation

The data used for analysis should be of sufficient quality and include relevant baseline data, to allow standard statistical procedures to be applied. Analysis of the data should be performed using validated statistical procedures. The results of such statistical analyses should allow subsequent decisions to be formulated on a sound scientific basis. Furthermore, these analyses should indicate whether the applied sampling and data collection strategies were correct or need to be modified. In cases where adverse effects are identified, it must be clearly distinguished if these effects were caused by the release of the GMO or by other factors. If this is uncertain, further assessments should be performed to clarify this issue.

The results obtained by the data evaluation procedure should be usable in decision-making processes. These include decisions concerning the validity of the ERA and risk management, decisions on renewal or withdrawal of the approval for market release of the GMO, and decisions on countermeasures against adverse effects. As already mentioned, the connection between the results obtained by monitoring and the resulting options and triggers for decision-making need to be verified before commencing any monitoring activity.

4.6.2 Data reporting and data storage

DATA REPORTING
The ability to base decisions on the monitoring data is inherently linked to the reporting of the data. It needs to be ensured that data are communicated to all relevant stakeholders with an interest in agriculture and ecosystem function, relevant decision makers and the general public.

The ability to base decisions on the monitoring data is inherently linked to the reporting of the data. It needs to be ensured that data are communicated to all relevant stakeholders with an interest in agriculture and ecosystem function, relevant decision-makers and the general public. The availability of competent personnel who are capable of translating scientific research findings obtained by the monitoring procedure into a common language is important in this respect. Transparency of the entire monitoring process and subsequent decision-making processes need to be ensured. Methods for communicating and publishing monitoring results could include (EU, 2002b):

- information sheets distributed to users and stakeholders;
- presentation and exchange of information with stakeholders during workshops;
- publication of information in relevant media, e.g. scientific journals;
- archiving of information by the company responsible for the GMO or the responsible competent authority;
- availability of information online, e.g. on company Web sites or Web sites of the responsible competent authority.

In addition, a national database comprising all information obtained from post-release monitoring could be established. Such a database could be used for centralized collection of data, providing processed information to stakeholders,

decision-makers and the general public, and facilitate the exchange of data (Wilhelm *et al.*, 2003). The database could also contain background information on the monitoring programmes. However, the establishment, maintenance and administration of such a database will require a certain amount of financial and personnel input.

4.6.3 Review of the monitoring plan

Following the first monitoring period, the obtained data should be used to review and analyse the monitoring programme itself. Following such an analysis, necessary adjustments or upgrades on the monitoring programme, the monitoring goals and the methodology can be performed. The effectiveness and efficiency of data collection and measurements should be evaluated, including the statistical methods used for data evaluation. Furthermore, it should be verified that the employed measures are effective at addressing the questions and goals of the monitoring programme. If models have been used for predictive purposes and the formulation of hypotheses to be tested, these models should be evaluated and compared with the collected data. Progress and new developments in methods for data collection and measurement should also be incorporated when revising and updating a monitoring programme. In addition to the monitoring plan, the ERA for a given GMO should also be revised and updated using the information generated by the monitoring programme.

4.7 CRITICAL CONSIDERATIONS AND PROBLEMS

A basic goal of monitoring is to create knowledge necessary for the protection of agrosystems, rural livelihoods, human and animal health, and environmental and ecological integrity. Monitoring should be a goal-oriented process, with the aim of identifying and quantifying the effects that a GMO release has on selected agro- and ecosystem parameters; it is not a broad environmental research programme.

Monitoring should address the priorities of all stakeholders concerned with the process; the connection between the results obtained through monitoring and their impact on subsequent decision-making should be clearly defined. This requires precise formulation of goals and questions to be investigated, careful planning of the process, early and continous involvement of stakeholders, and the definition of triggers for decision-making (FAO, 2005).

A major challenge for monitoring is the large variation between agro-ecosystems, individual crop types and their interaction with the environment. Therefore, monitoring programmes need to be designed with regard to the local context and the individual GMO in order to obtain significant and valuable results. Furthermore, even clear effects might be difficult to quantify due to the complexity of agro-ecosystems, and agriculture in itself generates strong ecological signals. Therefore, care must be taken to design monitoring programme so that effects can be detected above the ecological "noise" produced by agriculture, and that a clear cause can be assigned to such effects – i.e. if they are caused by the GMO or not (Jepson, 2005). The careful choice of monitoring indicators and the availability of long-term baseline data and negative controls are critical in this respect.

Another point that needs to be taken into consideration when planning a post-release monitoring programme is the availability of financial resources, infrastructure and trained personnel. The scale of the monitoring programme should be adapted to the available resources, and the costs of monitoring should be in relation to the potential value of the GMO and the consequences of potential adverse effects. Maintaining a correct balance between sound science and practicability in terms of cost and other resources should be aimed at (Bartsch, 2005). An efficient coordination and splitting of tasks between all parties involved in the monitoring process is recommended in order to render the process as effective as possible. Harmonizing and standardizing GMO monitoring procedures and criteria and establishing good monitoring practices will be helpful

REVIEW OF THE MONITORING PLAN

Following the first monitoring period, the obtained data should be used to review and analyse the monitoring programme itself. Following such an analysis, necessary adjustments or upgrades on the monitoring programme, the monitoring goals and the methodology can be performed.

in this respect, for example by systematic and consecutive documentation of monitoring programmes and the establishment of methodological handbooks (Wilhelm *et al.*, 2003).

A summary of recommendations and guidance for scientists, the international community, policy- and decision-makers and international organizations concerning all aspects of monitoring can be found in FAO, 2005. This publication also contains two monitoring programme design templates addressing all relevant points and including relevant case examples, one for developed countries with sufficient knowledge and resources to carry out detailed monitoring programmes, and one for countries with limited experience, information and resources available.

CHAPTER 5

GMO TRACEABILITY AND LABELLING - A NEED FOR COMMERIAL MONITORING

Traceability can be defined as the ability to trace GMOs and products derived from GMOs throughout all stages of the placing on the market, i.e. through all production and distribution chains and networks. Traceability and correct labelling of approved GMOs and products derived from them need to be ensured at all stages of commercial release and placing on the market. Such requirements for traceability of GMOs and correct labelling will ensure that products can be easily withdrawn from the market in case unforeseen adverse effects on human health or the environment are found. Furthermore, traceability will allow targeted monitoring for potential effects of the GMO, and facilitate the implementation of risk management measures (EU, 2003b).

TRACEABILITY
Traceability can be defined as the ability to trace GMOs and products derived from GMOs throughout all stages of the placing on the market, i.e. through all production and distribution chains and networks.

Another important aspect of efficient traceability and labelling systems is the provision of correct and accurate information to every person involved in the trade and marketing of GMOs, and especially to the final consumer. Detailed, complete and reliable information regarding GMOs and derived products will allow consumers to make informed and free product choices.

The more complex the production chain network is, the more difficult it becomes to trace individual products or components of products. Tracing becomes even more difficult in production chain networks with extensive product branching, or with continuous rather than batch production methods. At present, GMO traceability and labelling systems are not being adequately implemented and monitored. Monitoring is only practised for certain *Identity Preservation* systems, representing only a very small proportion (< 1 percent) of the total market. Detailed, clear and feasible provisions and instructions should be given for implementing and monitoring GMO traceability and labelling systems. Steps towards effective and reliable traceability and labelling systems could include:

- the assignment of a simple numeric or alphanumeric code (Unique Identifier) to each single GMO, allowing fast identification of the GMO and retrieval of specific information about that GMO;
- clear and reliable transmission of information, from each stage of market placing or production chain to the next, that the material contains or consists of GMOs; provision of the unique identifier, if available;
- for processed products, an indication of each of the ingredients which is produced from GMOs;
- for pre-packaged products available to the final consumer, a clear notification that the product contains or consists of GMOs should be placed on the label;
- for non pre-packaged products available to the final consumer, a clear notification that the product contains or consists of GMOs should appear in connection with the display of the product.

THRESHOLD VALUES
For labelling purposes, it is recommended that threshold values for the presence of GMO material in food or other products be defined, which, in case they are exceeded, require appropriate labelling of the product.

In many cases, traces of GMO material in processed products may be adventitious or technically unavoidable due to the production and processing processes. In such cases, no traceability and labelling requirements should come into force. However, defined **threshold values** for the presence of adventitious or technically unavoidable GMO material in products should be set. Compliance with such threshold

values should be regularly controlled by adequate GMO detection and quantification techniques (see Module A: Agricultural Biotechnology). If the set threshold value is exceeded, the presence of the GMO material needs to be indicated on the label of the product. Furthermore, only the adventitious or technically unavoidable presence of approved GMOs should be tolerated; material from GMOs that have not received approval for commercial release and placing on the market must not be contained in any products placed on the market and available to consumers.

It is recommended that the responsible competent authority for traceability and labelling requirements regularly perform inspections and controls to check for compliance with traceability and labelling requirements. Several testing methods to detect and quantify GMO material in different samples, both raw material and processed products, exist and should be employed for such inspections and controls (see Module A: Agricultural Biotechnology). Furthermore, it is recommended that information on all stages and transactions performed during placing on the market and processing of a product containing GMO material be recorded and kept for an appropriate time period (e.g. five years in EU legislation) by the person performing such operations. Compliance with such information holding requirements could also be verified by the responsible competent authority.

A detailed discussion on traceability and labelling, focusing on the legal background and relevant international legislative documents, can be found in Module E: Legal Aspects.

CHAPTER 6

MONITORING GMO IMPORTS AND TRANSBOUNDARY MOVEMENTS

MONITORING GMO IMPORTS
Monitoring and controlling imports of GMOs or derived material is an important aspect associated with the commercial release of a GMO.

Worldwide plant quarantine is a legal enforcement measure aimed at preventing pests and pathogens from spreading or, in case these have already found entry and have established in a restricted area, preventing these from multiplying further. The same procedure should be extended to imported GMOs or GMO products from a foreign source which are destined for release within the importing country. There is a need for controlled testing of GMO material in a containment facility prior to release into the environment in order to identify and avoid its potential risks to human health and the environment. Therefore, monitoring the import, the quarantine procedure and post-quarantine handling/movement of the GMOs is crucial to regulate and implement proper application and deployment of GMOs and prevent any form of unintended biosafety regulation violations or oversight.

6.1 IMPORT PROCEDURES AND INFORMATION REQUIREMENTS

The information that should be collected and collated by the exporter prior to any export of GMO or GMO material, and which should be carefully checked by the importer, is listed in Annex 10. The individual steps that should be followed during the export/import procedure by the importing country are delineated and explained in detail below:

a. Collection and verification of adequate information on the nature of the transgene and its expression characteristics in the host organism

The importing institution/organization should be fully aware of the nature of the transgene, its source of origin (bacterial, animal/insect, plant), hazards/risks associated with it and the final expression product(s) of the transgene in the specific host organism.

b. Receive clearance from the GMO regulatory authority in the GMO receiving (importing) country

The statutory GMO regulatory authority is required to clear the import proposal of the GMO or GMO material after assessing:

- the purpose behind the import;
- the product(s) of the transgene with reference to the targeted ecological area;
- detection methodologies employed and validated for detecting the presence of the transgene in the GMO or derived material;
- all information regarding the toxicity/allergenicity/other effects of the transgene product;
- characteristics of transgene expression in the host organism;
- biochemical/physiological consequences or output of the transgene product(s) in the host organism;
- altered characteristics of the host organism due to transgene expression;
- research/commercial permit that the GMO or GMO material has been granted in the exporting country;
- any intellectual property rights regulations connected with the transgene, the GMO or GMO material restricting use in the importing country;
- potential utility and benefits of the transgene and the resulting GMO.

c. Award of the import permit and authorized import accompanied by a phytosanitary certification

For efficient monitoring of imports, it is recommended that a single competent authority (with multiple terminals in the case of large countries) be authorized

to award an import licence. Specific attention should be paid to the existence of earlier imports of the material and the concomitant assignment of an accession number/unique identifier (see below). If there are multiple agencies authorized to award import licences, documentation of the incoming material and assignment of accession numbers can become unsystematic and redundant, thus making monitoring of imports a difficult as well as expensive task. During the import process of the GMO or GMO material, it should be accompanied by the original import permit and the phytosanitary certificate (in the case of plants) from the country of export.

d. Documentation of the national accession number/unique identifier after entry into the importing country

ACCESSION NUMBER/UNIQUE IDENTIFIER
It is recommended that a specific accession number or unique identifier be assigned to each GMO in order to facilitate traceability and tracking of all operations performed with that GMO.

A data bank of all imports should be maintained with complete documentation regarding the material being imported. This will facilitate the evaluation of material in quarantine facilities if similar material has a history of import and quarantine processing. The potential risks can be directly associated with the foreign transgene and the host organism. Assigning a specific **accession number/unique identifier** to every GMO or GMO material entry has to be done carefully in order to prevent any duplication in case the material was already imported earlier. The accession number/unique identifier of the material should be stated as reference for every utilization, deployment or processing of the specific GMO material in the country of import. This will allow fast retrieval of relevant information about the GMO at every stage of GMO usage and by every person involved in any GMO operation (see EU, 2004 as an example).

e. Quarantine processing

Once the GMO or GMO material has received an accession number/unique identifier by the importing country, the material is passed through quarantine filters and procedures. Recommendations of the GMO regulatory authority on the GMO or GMO material that were made while granting the clearance of the import proposal should be taken into consideration for planning and conducting the quarantine procedures.

After passing through the routine quarantine processes the GMO should be kept under containment for a specified time period, depending on the individual GMO (for plants, one reproductive cycle, i.e. one growing season is recommended). During the contained growth, the GMO and derived material are subjected to:

- detection of the transgene that it is documented to be carrying and analysis of the expression characteristics;
- testing for any non-target trait expression of unusual or hazardous nature including pathological indications;
- testing of harvested seed for genetic use restriction technologies (GURT);
- analysis for phytosanitory aspects, i.e. if the GMO and derived material are harbouring any diseases or presents any other relevant phytosanitary hazard (in case of plants).

Following such careful experimental analyses of the imported GMO and verification of the characteristics and specifications of the GMO provided by the exporter, the GMO and derived material should be approved for release in the importing country. However, if any of the provided information is found to be incorrect or any other deviations regarding the characteristics of the GMO are detected, approval should not be granted. In such a case, it is recommended that clarification of the issue be requested from the exporter, and that the impact of the identified deviations of the GMO be analysed further. Specifically, the impact of any detected deviations on the risk assessment of the GMO, i.e. if they represent any form of risk in the context of the importing country and the conditions of the anticipated release, should be carefully assessed.

f. Recording and sample storage of imported GMOs

It is recommended that a "gene bank" of imported GMO material be developed, i.e. a facility to store references of GMO material that has been imported for prolonged periods of time. The samples should be maintained both as viable material and as isolated DNA containing the transgene as extracted from the imported material.

6.2 POST-QUARANTINE HANDLING AND MONITORING OF THE GMO

The competent authority responsible for GMO monitoring should review the import procedure both at the site and time of import as well as during quarantine and post-quarantine processing to ensure compliance with relevant legislation and procedure recommendations. The indicators for monitoring those procedures could be, amongst others:

- the permit for legal entry of the imported material;
- the accompanying phytosanitary certification from the source (exporting) country;
- detection of the transgene in the GMO material imported during quarantine;
- evaluation of the imported GMO material under containment for the recommended time period, including progeny analysis of the imported material and presence of marker genes the material is known to possess;
- correct handling of the GMO material and checking the biosafety level it is grouped in, for work within the quarantine containment facility;
- documentation and maintenance of the DNA from the imported material, with reference to the transgene detected and storage as national referral sample; these reference samples can also be important as standards for comparison in the post-release monitoring process of the imported GMO material.

6.3 FURTHER RECOMMENDATIONS FOR GMO TRANSBOUNDARY MOVEMENT

Efficient supervision and control of transboundary movements of GMOs is recommended in order to limit the potential risks associated with the release of GMOs and allow consumers to make free and informed choices regarding GMOs and derived material. In this respect, the establishment of legal frameworks regulating import, export and transboundary movement of GMOs and derived materials is

recommended. Information plays a critical role in those processes; efficient coordination and sharing of all relevant information concerning a GMO between exporting and importing parties are required in order to allow the parties to make informed decisions on any import/export processes (see Annex 10). Ensuring this is especially important in developing countries, where institutional and/or human capacities to evaluate import/export processes might be limited. In addition to providing and exchanging information prior to import/export activities, relevant information documents should also accompany GMOs and GMO derived material during the import/export and transboundary movement processes; the list provided in Annex 10 can also be used as guidance in this respect.

One international document that extensively addresses the issue of transboundary movement and related problems of GMOs is the Cartagena Protocol on Biosafety (CPB) (CBD, 2000). Please refer to Module E: Legal Aspects for a detailed introduction to the topic.

In the event of an unintentional release in a state of a GMO that has potential adverse effects on human health and the environment, and this release leads to unintentional transboundary movement of the GMO to neighbouring states, the responsible national competent authority should take appropriate measures. Such measures include providing information to the public, affected or potentially affected states, the Biosafety Clearing House created under the CPB and relevant international organizations. Providing detailed information about the GMO and the details of the unintentional release will allow fast and appropriate responses and the implementation of measures to limit the risks posed by the GMO (EU, 2003a).

GMO TRANSBOUNDARY MOVEMENT

Efficient supervision and control of transboundary movements of GMOs is recommended in order to limit the potential risks associated with the release of GMOs and allow consumers to make free and informed choices regarding GMOs and derived material. In this respect, the establishment of legal frameworks regulating import, export and transboundary movement of GMOs and derived materials is recommended.

ANNEX 1

RISK ASSESSMENT PARAMETERS AND PROCEDURES FOR GMMS

The following parameters should be taken into account during the risk classification procedure for a GMM operation and result in a classification of the operation into one of the four risk classes. Special attention should be paid to the following harmful effects (EU, 1998):

- diseases to humans including allergenic or toxic effects;
- diseases to plants and animals;
- deleterious effects due to the impossibility of treating a disease or providing an effective prophylaxis;
- deleterious effects due to establishment or dissemination in the environment;
- deleterious effects due to the natural transfer of inserted genetic material to other organisms.

The assessment should be based on the following key points (EU, 1998):

- the identification of any potentially harmful effects, in particular those associated with:
  - the recipient micro-organism;
  - the genetic material inserted (originating from the donor organism);
  - the vector;
  - the donor micro-organism (as long as the donor micro-organism is used during the operation);
  - the resulting GMM;

- the characteristics of the activity;
- the severity of the potentially harmful effects;
- the likelihood of the potentially harmful effects being realized.

The detailed list of parameters recommended for the assessment is provided below (extracted from EU, 1990), structured into thematic groups A to D:

A. Characteristics of the donor, recipient or (where appropriate) parental organism(s)
B. Characteristics of the modified micro-organism
C. Health considerations
D. Environmental considerations

A. Characteristics of the donor, recipient or (where appropriate) parental organism(s)

- name and designation;
- degree of relatedness;
- sources of the organism(s);
- information on reproductive cycles (sexual/asexual) of the parental organism(s) or, where applicable, of the recipient micro-organism;
- history of prior genetic manipulations;
- stability of parental or of recipient organism in terms of relevant genetic traits;
- nature of pathogenicity and virulence, infectivity, toxicity and vectors of disease transmission;
- nature of indigenous vectors;
- DNA sequences;
- frequency of mobilization;
- specificity;
- presence of genes which confer resistance;
- host range;
- other potentially significant physiological traits;
- stability of these traits;

- natural habitat and geographic distribution; climatic characteristics of original habitats;
- significant involvement in environmental processes (such as nitrogen fixation or pH regulation);
- interaction with, and effects on, other organisms in the environment (including likely competitive or symbiotic properties);
- ability to form survival structures (such as spores or sclerotia).

B. Characteristics of the modified micro-organism

- the description of the modification including the method for introducing the vector insert into the recipient organism or the method used for achieving the genetic modification involved;
- the function of the genetic manipulation and/or of the new nucleic acid;
- nature and source of the vector;
- structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified micro-organism;
- stability of the micro-organism in terms of genetic traits;
- frequency of mobilization of inserted vector and/or genetic transfer capability;
- rate and level of expression of the new genetic material; method and sensitivity of measurement;
- activity of the expressed protein.

C. Health considerations

- toxic or allergenic effects of non-viable organisms and/or their metabolic products;
- product hazards;
- comparison of the modified micro-organism with the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
- capacity for colonization;
- if the micro-organism is pathogenic to humans who are immunocompetent:

a) diseases caused and mechanism of pathogenicity including invasiveness and virulence;
b) communicability;
c) infective dose;
d) host range, possibility of alteration;
e) possibility of survival outside of human host;
f) presence of vectors or means of dissemination;
g) biological stability;
h) antibiotic resistance patterns;
i) allergenicity;
j) availability of appropriate therapies.

D. Environmental considerations

- factors affecting survival, multiplication and dissemination of the modified micro-organism in the environment;
- available techniques for detection, identification and monitoring of the modified micro-organism;
- available techniques for detecting transfer of the new genetic material to other organisms;
- known and predicted habitats of the modified micro-organism;
- description of ecosystems into which the micro-organism could be accidentally disseminated;
- anticipated mechanism and result of interaction between the modified micro-organism and the organisms or micro-organisms which might be exposed in case of release into the environment;
- known or predicted effects on plants and animals such as pathogenicity, infectivity, toxicity, virulence, vector of pathogen, allergenicity, colonization;
- known or predicted involvement in biogeochemical processes;
- availability of methods for decontamination of the area in case of release into the environment.

Ultimately, careful evaluation of these parameters and, possibly, additional consultation of relevant background literature and risk classification manuals (e.g. WHO, 2004; NIH, 2009) should allow the risk classification of the GMM operation. This risk classification then allows the appropriate containment level and the containment structures that are required to guarantee safe working procedures and protection of human health and the environment to be determined.

The ultimate assignment of a containment level could be further influenced by the following considerations:

- the characteristics of the environment likely to be exposed (e.g. whether in the environment likely to be exposed to the GMMs there are known biota which can be adversely affected by the micro-organisms used in the contained use activity);
- the characteristics of the activity (e.g. its scale; nature);
- any non-standard operations (e.g. the inoculation of animals with GMMs; equipment likely to generate aerosols).

An assessment of the above points could lead to a change in the level of risk assigned to the GMM operation, and similarly to the containment level required for that operation (lowering, increment or no effect).

ANNEX 2

CONTAINMENT MEASURES (BIOSAFETY LEVELS) FOR LABORATORIES PERFORMING GMM OPERATIONS

The following table was adapted from Health and Safety Executive, 2007. Different requirements exist for large-scale operations involving GMMs, operations involving GMMs and animals, and operations involving GMMs and plants; please refer to the Health and Safety Executive publication or similar publications (e.g. NIH, 2009; WHO, 2004) for detailed lists of the relevant containment requirements. Detailed annotations of how to comply with the individual points in the table are also included in those publications.

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
Laboratory suite isolation	not required	not required	required	required
Laboratory suitable for fumigation	not required	not required	required	required
EQUIPMENT				
Surface impervious to water and resistance to acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	required for bench	required for bench	required for bench and floor	required for bench, floor, ceiling and walls

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
Entry to laboratory via airlock	not required	not required	required where and to extent the risk assessment shows it is required	required
Negative pressure relative to the pressure of the immediate surroundings	not required	required where and to extent the risk assessment shows it is required	required	required
Extract and input air from the laboratory should be HEPA filtered	not required	not required	HEPA filters required for extract air	HEPA filters required for input and extract air
Microbiological safety cabinet/enclosure	not required	required where and to extent the risk assessment shows it is required	required and all procedures with infective materials required to be contained within a cabinet/ enclosure	Class III cabinet required
Autoclave	required on site	required in the building	required in the laboratory suite	double ended autoclave required in laboratory
SYSTEM OF WORK				
Access restricted to authorized personnel only	not required	required	required	required via airlock key procedure
Specific measures to control aerosol dissemination	not required	required so as to minimize	required so as to prevent	required so as to prevent
Shower	not required	not required	required where and to extent the risk assessment shows it is required	required

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
Protective clothing	suitable protective clothing required	suitable protective clothing required	suitable protective clothing required; footwear required and to extent the risk assessment shows it is required	complete change of clothing and footwear required before entry and exit
Gloves	not required	required where and to extent the risk assessment shows it is required	required	required
Efficient control of disease vectors (e.g. for rodents and insects) which could disseminate the GMM	required where and to extent the risk assessment shows it is required	required	required	required
Specified disinfection procedures in place	required where and to extent the risk assessment shows it is required	required	required	required
WASTE				
Inactivation of GMMs in effluent from hand washing sinks and showers and similar effluents	not required	not required	required and to extent the risk assessment shows it is required	required
Inactivation of GMMs in contaminated material	required by validated means	required by validated means	required by validated means, with waste inactivated in the laboratory suite	required by validated means, with waste inactivated within the laboratory

CONTAINMENT MEASURES	CONTAINMENT LEVEL			
	1	2	3	4
OTHER MEASURES				
Laboratory to contain its own equipment	not required	not required	required so far as is reasonably practicable	required
An observation window or alternative is to be present so that occupants can be seen	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required	required
Safe storage of GMMs	required where and to extent the risk assessment shows it is required	required	required	secure storage required
Written records of staff training	not required	required where and to extent the risk assessment shows it is required	required	required

ANNEX 3

GOOD LABORATORY PRACTICE

The following points should be considered for every operation with GMOs and within containment facilities (EU, 1998; see also WHO, 2004):

- to keep workplace and environmental exposure to any GMM to the lowest practicable level;
- to exercise engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment when necessary;
- to test adequately and maintain control measures and equipment;
- to test, when necessary, for the presence of viable process organisms outside the primary physical containment;
- to provide appropriate training of personnel;
- to establish biological safety committees or subcommittees, if required;
- to formulate and implement local codes of practice for the safety of personnel, as required;
- where appropriate to display biohazard signs;
- to provide washing and decontamination facilities for personnel;
- to keep adequate records;
- to prohibit eating, drinking, smoking, applying cosmetics or the storing of food for human consumption in the work area;

- to prohibit mouth pipetting;
- to provide written standard operating procedures, where appropriate, to ensure safety;
- to have effective disinfectants and specified disinfection procedures available in case of spillage of GMMs;
- to provide safe storage for contaminated laboratory equipment and materials, when appropriate.

In addition to these principles, the appropriate containment measures for the risk class of the operation should be in place in order to asssure protection of human health and the environment.

The containment measures applied shall be periodically reviewed by the user to take into account new scientific or technical knowledge relative to risk management and treatment and disposal of wastes.

ANNEX 4

RISK ASSESSMENT PARAMETERS AND PROCEDURES FOR GM PLANTS

The following parameters should be taken into account during the risk classification procedure for a GM plant operation and result in a classification of the operation into one of the four risk classes. The containment measures associated with each of the four risk classes (also referred to as biosafety levels) should be sufficient to control all potential harmful effects of the organisms assigned to a risk class and provide sufficient protection for human health and the environment. The risk assessment procedure can be divided into two parts: a risk assessment for the environment, and a risk assessment for human health. The risk assessment should also take into account the nature of the work, for example, large-scale operations, non-standard operations or non-standard growth facilities (tanks or fermenters for algae, cages for GM trees, etc).

Risk assessment for the environment:

Potential hazards to be considered include:

- the ability of the GM plant to survive, establish and disseminate in the receiving environment;
- hazards associated with the inserted transgene;
- the potential for transfer of the transgene between the GM plant and other organisms;
- phenotypic and genetic stability of the genetic modification.

In detail, the points to be evaluated include (adapted from Health and Safety Executive, 2007):

- the ability of the GM plant to survive and reproduce in the receiving environment;
- the ability of the GM plant to establish, i.e. to colonize habitats and compete with native species (invasiveness);
- enhanced competitiveness of the GM plant compared with other plant species or the unmodified species (weediness);
- the ability of the GM plant to form survival structures (e.g. seeds) and the distance over which they are distributed;
- the ability of a GM plant to cause harm even if it is unable to survive, e.g. by gene transfer;
- the potential of a GM plant to cause adverse effects on organisms in the receiving environment due to the expression of the transgene (nature of the transgene and expressed proteins);
- the ability to cause harm to plants, e.g. by root exudates;
- the ability to cause harm to animals, e.g. by toxic or allergenic expression products;
- the ability to cause harm to beneficial mirco-organisms in the soil or water, e.g. by expression of anti-fungal proteins;
- the ability to cause harm to non-target organisms, e.g. expressing pest-resistance traits that affect a broad range of non-target organisms;
- the possibility of virus transencapsidation, if the transgene codes for a viral coat protein;
- the possibility of recombination between the mRNA of the transgene with the RNA genome of a plant virus;
- the possibility of synergistic effects, e.g. between an infecting virus and an expressed viral coat protein;
- the properties of the transgene product in combination with the expression characteristics, i.e. the temporal and spatial expression profile of toxic or allergenic transgene products;

- verify the genetic and phenotypic stability of the transgene over several generations, e.g. investigate the amount of gene silencing;
- evaluate the possibilities for transgene transfer between the GM plant and other organisms;
- evaluate the possibilities for pollen transfer and outcrossing with related, compatible species;
- special attention should be paid to novel genes, e.g. transgenes coding for biologically active compounds (biopharming).

Risk assessment for human health:

- Nature of the transgene/the expressed proteins: toxic or allergenic effects on humans? Expression of biologically active compounds, e.g. vaccines or other pharmaceutical compounds (biopharming)?
- Possible routes of exposure to transgenic plant material; indirect (e.g. pollen via air), direct contact or ingestion required to obtain adverse effects?

Following the evaluation of these factors, the likelihood of identified potential hazards being realized should be assessed. This can be a difficult process, however several indicators might facilitate this evaluation. For example, specific parameters, obtained by laboratory testing, can be assigned to many processes, such as typical frequencies for hybridization, pollen dispersal ranges, survival rates of the non-modified parent organism in the receiving environment, etc. Characteristics of the receiving environment that either support or restrict potential adverse effects are especially important in this evaluation. A final assessment should classify the likelihood of adverse effects being realized from "negligible" to "high".

Following the assessment of likelihood, the severity of the potential consequences of each hazard should be assessed, again using a classification from "negligible" to "high". Combining the likelihood of a hazard with its consequences yields the

final risk classification (see Module C: Risk Analysis). A precautionary approach should be applied to both the assessment of likelihood and the consequences: when the level of knowledge is insufficient to establish a classification with certainty, a higher level should be employed. The final risk level then defines the containment measures that are required to reduce the risks to "low or effectively zero" (Health and Safety Executive, 2007); the containment measures for the four plant risk classes are provided in Annex 5.

ANNEX 5

CONTAINMENT MEASURES (BIOSAFETY LEVELS) FOR GREENHOUSE ACTIVITIES WITH GM PLANTS

The following containment measures relating to the four biosafety levels for plants (BL1-P to BL4-P) were extracted from the NIH Guidelines (NIH, 2009). Please refer to this or similar publications (e.g. WHO, 2004) for detailed descriptions of the individual containment measures and background information. Where research involving both plants and micro-organisms is performed, the containment measures for GMMs should also be taken into consideration (Annex 2).

In addition to the containment measures listed below, the standards of good laboratory practice (Annex 3) should be followed at all times.

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
GREENHOUSE ACCESS:				
Limited or restricted	Yes	Yes	Yes	Yes
Access managed by responsible individual	/	/	/	Yes, access through secure, locked doors
Warning of potential hazards prior to entering	/	/	/	Yes
Entrance only through clothing change and shower room	/	/	/	Yes, shower each time greenhouse is left
Training prior to access	Yes	Yes	Yes	Yes

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
RECORDS:				
Record of current experiments	Yes	Yes	Yes	Yes
Record of all organisms that are brought into or removed from the greenhouse	/	Yes	Yes	Yes, plus of all materials
Reporting of any accident involving release of GMOs	/	Yes	Yes	Yes
Record of persons entering/ exiting the greenhouse	/	/	/	Yes
DECONTAMINATION AND INACTIVATION:				
GMOs rendered biologically inactive before disposal	Yes	Yes	Yes, autoclaving recommended	Yes, by autoclaving
Decontamination of run-off water	/	Recommended	Yes	Yes
Decontamination of equipment	/	/	Yes	Yes
CONTROL OF UNDESIRED SPECIES:				
Programme to control undesired species	Yes	Yes	Yes	Yes, chemical control
Anthropods and motile macro-organisms kept in cages; precautions to minimize escape	Yes	Yes	Yes	Yes
CONCURRENT EXPERIMENTS CONDUCTED:				
Experiments with a lower biosafety level can be conducted concurrently	Yes	Yes	Yes	Yes
GREENHOUSE DESIGN:				
Greenhouse floor	Gravel or other porous material	Impervious material. Gravel under benches and soil beds acceptable.	Impervious material with collection of run-off water	Walls, roof and floor form sealed, resistant internal shell
Windows and wall/roof openings	May be open for ventilation	May be open for ventilation	Closed and sealed	Closed and sealed
Glazing	/	/	Resistant to breakage	Resistant to breakage
Screens	Recommended	Required	/	/
Greenhouse isolation and entry	/	/	Closed self-contained structure, self-closing locking doors	Closed, self-contained structure, self-closing locking doors

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
Fencing and security	/	/	Yes	Yes
Internal walls, ceilings and floors	/	/	Resistant to penetration	Resistant to penetration
Benchtop material	/	/	Impervious, resistant surfaces	Impervious, resistant surfaces
Hand washing sink/shower	/	/	Sink, automatically operated	Shower
Changing rooms	/	/	/	Yes, outer and inner and shower
Airlock	/	/	/	Yes, for material passage
AUTOCLAVES:				
An autoclave should be available	/	Yes	Yes	Yes, double-door
Air ventilation systems:				
Minimize entrance of anthropods	/	Yes	/	/
Individual supply and exhaust systems	/	/	Yes	Yes
Negative pressure	/	/	Yes	Yes
HEPA filtering of exhaust air	/	/	Yes	Yes
HEPA filtering of ventilation lines	/	/	Yes, on vacuum lines	Yes
SIGNS:				
Signs indicating that a restricted experiment is in progress	/	Yes	Yes	Yes
Signs indicating the presence of organisms with potential for environmental damage	/	Yes, if applicable	Yes, if applicable	Yes, if applicable
Sign indicating risks to human health (biohazard sign)	/	Yes, if applicable	Yes, if applicable	Yes, if applicable
TRANSFER OF MATERIALS:				
Transfer of viable organisms to/from the facility	/	Transfer in a closed, non-breakable container	Transfer in a sealed secondary container	Transfer in a sealed secondary container

CONTAINMENT MEASURES	CONTAINMENT LEVELS			
	1	2	3	4
Transfer of materials and supplies	/	/	/	Transfer through autoclave airlock or fumigation chamber
PROTECTIVE CLOTHING:				
Disposable clothing should be worn in the greenhouse	/	/	Yes, if considered necessary	Yes, may be disposable
Exchange of street clothing to complete laboratory clothing	/	/	/	Yes
Protective clothing removed before exiting the greenhouse and decontaminated	/	/	Yes	Yes, by autoclaving
GREENHOUSE PRACTICES MANUAL:				
A greenhouse practices manual should be prepared and adopted	/	Yes	Yes	Yes
OTHER:				
Hand wash upon exiting the greenhouse	/	/	Yes	/
Shower upon exit	/	/	/	Yes
Procedures performed to minimize creation of aerosols/splashes	/	/	Yes	Yes

ANNEX 6

RISK ASSESSMENT PARAMETERS AND PROCEDURES FOR GM ANIMALS

The risk assessment process for GM animals is essentially the same as already described for GM micro-organisms and GM plants in Annexes 1 and 4, respectively. Again, the risk assessment procedure can be divided in a risk assessment for the environment and a risk assessment for human health. Points to evaluate include:

Risk assessment for the environment:

- ability of the GM animal to survive in the receiving environment;
- adverse effects if the GM animal cannot establish, but is able to survive in the short term;
- interactions of the GM animal in the receiving environment, e.g. displacement of or competition with native species, prey upon native species (including plants) and physical damage, including all direct and indirect implications for ecosystem function;
- effects of the genetic modification on the animal's survivability and niche range (e.g. increased tolerance to environmental conditions or increased fecundity);
- feasibility of recovering escaped individuals;
- expression of biologically active compounds (biopharming) and effects on interacting species;
- potential of the GM animal to act as a novel animal disease vector or reservoir;

- transfer of transgenes to other species in the receiving environment; presence of sexually compatible species;
- the nature of the transgene with regard to possible transgene transfer: if it confers a selective advantage or disadvantage;
- transgene stability and possible transgene loss with subsequent effects.

Risk assessment for human health:

- nature of the transgene and expressed protein: possible toxic or allergenic effects, bioactive compounds;
- GM animals acting as vectors or reservoirs for human diseases due to the genetic modification;
- altered behaviour of the GM animal, e.g. enhanced aggressiveness;
- general risk for human health arising from animal handling that might be influenced by the genetic modification, e.g. bites, scratches, zoonotic infections or allergenic reactions.

For further discussion of the individual points, please refer to Health and Safety Executive, 2007.

Following the hazard identification procedure, an assessment of the likelihood of these hazards being realized, as well as an assessment of the consequences in case the hazards are realized, is performed. This allows the establishment of a final risk classification and the grouping of the GM animal operation into one of four risk classes (biosafety levels). The characteristics of the receiving environment as well as the scale and nature of the GM animal operation are critical parameters in these assessments and require special consideration.

The containment measures for biosafety levels 1 to 4 for GM animals, which are required to reduce the risks to human health and the environment to low or effectively zero, are listed below in Annex 7.

ANNEX 7

CONTAINMENT MEASURES (BIOSAFETY LEVELS) FOR GM ANIMALS

In addition to these general biosafety requirements (extracted from NIH, 2009; please refer to that publication for details) special recommendations concerning the housing of specific groups of organisms (large and small mammals, aquatic animals, insects, etc.) exist. Details can be found in relevant guidance documents, see for example Health and Safety Executive, 2007; WHO, 2004.

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
ANIMAL FACILITY:				
Animals contained in enclosed structure (animal room)	Yes	Yes	Yes	Yes
Interior walls, floors and ceilings impervious and resistant	/	Yes	Yes	Yes
Windows	/	Fitted with fly screens	Closed, sealed, breakage resistant	Closed, sealed, breakage resistant
Autoclave available	/	Yes	Yes	Yes, or incinerator
Self-closing doors	/	/	Yes	Yes
Anthropod-proof structure	/	Yes	Yes	Yes
Double barrier between containment area and environment	/	/	Yes	Yes, animal area separated from all other areas

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
Necropsy room	/	/	/	Yes
Decontamination of waste and run-off water	/	/	Yes	Yes, by heat or chemical methods
Directional airflow (inwards)	/	/	Yes	Yes
Double HEPA filtering of exhaust air	/	/	Single filter, if required	Yes
Exhaust air incinerator	/	/	/	Yes, as alternative to double HEPA filtering
Floor drains with deep traps	/	/	/	Yes
Hand washing sink	/	/	/	Yes, automatically operated
Restraining devices for animals	/	/	/	Yes
Supply water system with backflow preventer	/	/	/	Yes
All utilities, liquid and gas services with backflow preventer	/	/	/	Yes
Ventilation lines with HEPA filters	/	/	/	Yes
ANIMAL FACILITY ACCESS:				
Individuals under 16 years not permitted	/	/	/	Yes
Containment area locked	Yes	Yes	Yes	Yes
Containment area patrolled or monitored	Yes	Yes	Yes	Yes
Containment building patrolled, with locking access	/	Yes	Yes	Yes
Restricted access, warning of potential hazards	Yes	Yes	Yes	Yes
Entrance/exit through clothing change/shower rooms	/	/	/	Yes

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
All closures closed when experiment in progress	/	/	Yes	Yes
DECONTAMINATION AND INACTIVATION:				
All wastes decontaminated	/	Yes	Yes	Yes
Work surfaces and equipment decontaminated after work	/	/	Yes	Yes
Removal of material	/	/	Special requirements	Only after autoclaving
Chemical disinfectant shower for ventilated suits	/	/	/	Yes, if such suits are required
Needles and syringes placed in puncture-resistant containers	/	Yes, and decontaminated	Yes, and decontaminated	Yes, and decontaminated
SIGNS:				
Biohazard sign if special provisions (e.g. vaccination) required for entry	/	Yes	Yes	Yes
PROTECTIVE CLOTHING:				
Complete change of street clothing to laboratory clothing	/	No, but laboratory coats and gloves required	Yes, special care to minimize skin contamination	Yes, entry/exit only through change and shower rooms
Decontamination of clothing	/	/	Yes	Yes
Ventilated positive pressure suit	/	/	/	If appropriate
Respiratory protection	/	/	Yes	Yes
Records:				
Records of animal use and disposal	/	/	Yes	Yes
Records of incidents and accidents	/	Yes	Yes	Yes
Record of baseline serum samples	/	Yes, if appropriate	Yes, if appropriate	Yes
Record of personnel entry/exit	/	/	/	Yes

CONTAINMENT MEASURE	CONTAINMENT LEVELS			
	1	2	3	4
TRANSFER OF MATERIALS:				
Decontamination of material before removal	/	Yes	Yes	Yes, by autoclaving or gaseous/vapour methods
Material container for transport	/	Primary and secondary container required	Primary and secondary container required	Primary and secondary container required
Entry of materials and supplies	/	/	/	Through double-door autoclave or airlock
OTHER:				
Mark all GM neonates within 72 hours after birth	Yes	Yes	Yes	Yes
Eating, drinking, smoking and applying cosmetics not permitted	/	Yes	Yes	Yes
Hand wash before exiting containment area	/	Yes	Yes, or showering	Showering required
Concurrent conduct of experiments with a lower BL	Yes	Yes	Yes	Yes
Animal areas cleaned daily	/	/	Yes	Yes
Minimize creation of aerosols	/	/	Yes	Yes
Separate male and female animals	Yes	Yes	Yes	Yes
Life support system for ventilated suits with alarms and backup air tanks	/	/	/	Yes, if such suits are required
Specifications for needles and syringes	/	Yes	Yes	Yes
Quarantine, isolation and medical care facility for personnel	/	/	/	Yes
Preparation and adoption of a biosafety manual	/	Yes	Yes	Yes
Vacuum lines protected with HEPA filters	/	/	Yes	Yes
Appropriate steps to prevent horizontal transmission	/	Yes	Yes	Yes

ANNEX 8

MINIMUM ISOLATION DISTANCES AND MONITORING FREQUENCY FOR CONFINED FIELD TRIALS

The following table, stating minimum isolation distances and monitoring frequencies for selected GM crops in confined field trials, was adapted from Adair and Irwin, 2008.

Table | **Minimum isolation distances, periods of post-harvest land use restriction, and minimum monitoring frequency for confined research field trials**

CROP	MINIMUM ISOLATION DISTANCE	PERIOD OF POST-HARVEST LAND USE RESTRICTION	MONITORING FREQUENCY	
			Trial period	Post-harvest period
Agrostis palustris Huds. (creeping bentgrass)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Beta vulgaris L. (sugar beet)	3 m and harvest before flowering	2 years	weekly	every 2 weeks
Brassica carinata A. Braun (Ethiopian mustard)	200 m from other *Brassica* spp. 50 m from weedy relatives	3 years	weekly	every 2 weeks
Brassica juncea L. (brown mustard)	200 m from other *Brassica* spp. 50 m from weedy relatives	5 years	weekly	every 2 weeks
Brassica napus L. (Argentine rape canola)	200 m from other *Brassica* spp. 50 m from weedy relatives	3 years	weekly	every 2 weeks
Brassica rapa L. (Polish rape canola)	400 m from other *Brassica rapa* 200 m from other *Brassica* spp. 50 m from weedy relatives	5 years	weekly	every 2 weeks
Capsicum annuum (pepper)	20 m	1 year	every 2 weeks	every 2 weeks
Carthamus tinctorius L. (safflower)	400 m	2 years	weekly	every 2 weeks

CROP	MINIMUM ISOLATION DISTANCE	PERIOD OF POST-HARVEST LAND USE RESTRICTION	MONITORING FREQUENCY	
			Trial period	Post-harvest period
Cucurbita pepo L. (squash)	650 m	1 year	weekly	every 2 weeks
Glycine max (L.) Merr. (soybean)	10 m	1 year	every 2 weeks	every 2 weeks
Helianthus annuus L. (sunflower)			weekly	every 2 weeks
Hordeum vulgare L. (barley)	10 m	2 years	every 2 weeks	every 2 weeks
Lens culinaris Medik (lentil)	10 m	1 year	every 2 weeks	every 2 weeks
Linum usitatissimum L. (flax)	10 m	2 years	weekly	weekly
Lolium perenne L. (perennial grass)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Lycopersicon esculentum Mill. (tomato)	20 m	1 year	weekly	every 2 weeks
Medicago sativa L. (alfalfa)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Nicotiana tabacum (tobacco)	400 m	1 year		
Phalaris canariensis L. (canary seed)	10 m	2 years	every 2 weeks	every 2 weeks
Picea spp. (spruce)	removal of seeds and pollen cones	2 years minimum	monthly, twice a week during cone formation	monthly
Pisum sativum L. (pea)	10 m	1 year	every 2 weeks	every 2 weeks
Populus spp. (poplar)	removal of inflorescences	3 years minimum	monthly, twice a week during flowering and budburst	monthly
Sinapis alba L. (white mustard)	400 m from other *S. alba* 50 m from other *Brassica* spp. and weedy relatives	5 years	weekly	every 2 weeks
Solanum tuberosum L. (potato)	one blank row (~ 1 metre)	2 years	weekly	every 2 weeks
Trifolium repens L. (white clover)	300 m (without cropping)	3 years	weekly, daily and every 3rd day	every 2 weeks
Triticum aestivum L. (wheat)	30 m	2 years	every 2 weeks	every 2 weeks
Vitis spp. (grapevine)	bagging of flowers	3 years minimum	monthly, weekly at pollen shed	monthly
Zea mays L. (corn)	200 m	1 year	weekly	every 2 weeks

ANNEX 9

EXAMPLES OF INSPECTION QUESTIONS/MONITORING INDICATORS FOR CONFINED FIELD TRIALS

The following points can be used as a checklist to verify the compliance of a confined field trial with basic confinement and biosafety requirements as described in the main text. They could be used either by trial managers (permit holders) themselves to verify if their management of a confined field trial is correct, or by the competent authorities to check if confined field trials are being performed according to the issued release permit. The list only provides examples and thus is not exhaustive and should be adapted, and possibly extended, according to the local conditions and requirements of a field trial on a case-by-case basis. Adapted from Gosh, 2002; Department of Biotechnology, 2006; APHIS, 2008.

- Were the competent authorities informed of the trial? Was a correct application handed in and a release permit issued?
- Do the shipping and packing containers used for this field trial meet the specifications in the release permit?
- Were packing and shipping materials used for this field trial cleaned out and disposed of to meet the release permit?
- Were transport and storage containers employed so as to fully contain the GMO material at the field trial location?
- Are seed bags, packages, pots or other containers used for the GMO material clearly and durably marked so that each individual GMO can be distinguished and identified by the permit holder throughout the field trial process?

- Was an up-to-date map of the field trial site prepared and supplied to the competent authority?
- Conduct of the trial: Is the trial being conducted according to the approved field design with the replications and plot size mentioned (with acreage at or below the area indicated in the release permit)?
- Isolation: Is the isolation distance around the experimental area maintained with no related species or varieties of the same species in the area?
- If border rows are present in the field trial site, are they grown to meet permit conditions?
- If flower removal was used to control reproduction, was the technique employed successfully and recorded?
- If flower bagging was used to control reproduction, was the technique employed successfully and recorded?
- If temporal isolation (flowering time) was used to control reproduction, was the technique employed successfully and recorded?
- Is the design and management of the outermost boundary of the field site(s) sufficient to assure segregation and confinement during all field operations and growth stages?
- Are photographs clearly documenting the isolation of the crop right through planting to harvesting and post harvest management of crop debris?
- Does the permit holder have monitoring and removal records for sexually compatible plants within the isolation area of the field trial?
- Are measures being taken to minimize or prevent expected human or animal incursions onto the field trial?
- Toxicity/allergenicity data: Is the evaluation of the impact of the transgene product for its likelihood of causing any allergies or toxicity based on the guidelines in use?
- Safe storage of harvested seed and salvaging any spill in the field: Is sufficient care taken to harvest as much seed as possible and no seed is spilled and left behind? What measures were taken?

- Were operations to dispose and devitalize the GMO material (including field trial borders) fully employed?
- Do records show that equipment used in this field trial meets the specifications for the frequency and type of cleaning required in the release permit?
- Maintenance of field data: Were entire experimental data maintained and recorded and supplied to the competent authority?
- Do descriptions or records demonstrate that the permit holder is monitoring for deleterious/negative effects expressed by the regulated crop on itself, other plants, non-target species, or the environment?
- Are all the safety guidelines with respect to the personnel working with the experimenters taken care of?
- Were any accidents encountered? How was the emergency attended to by the competent authorities? Were any accidents and the countermeasures taken clearly documented and reported?
- Was a logbook recording the entries of all persons into the trial site correctly maintained?
- Was any unknown pest, insect or pathogen harmful or otherwise noted on the transgenic crop? If so, was it brought to the notice of the competent authority? What was the action taken after the observation?

ANNEX 10

RECOMMENDATIONS FOR INFORMATION THAT SHOULD BE PROVIDED BY THE EXPORTING PARTY FOR TRANSBOUNDARY MOVEMENTS AND IMPORT OF GMOs OR GMO-DERIVED MATERIAL

The following list provides indications on information that should be collected and collated by the exporting party prior to any GMO export. The information should be made available to the importing party before commencing any intentional transboundary movements. This list shall serve as a guideline, and may be extended or modified in adaptation to national requirements and the specific context and local conditions for import/export. Adapted from EU, 2003a.

- Name, address and contact details of the exporter.
- Name, address and contact details of the importer.
- Name and identity of the GMO, as well as the domestic classification, if any, of the biosafety level of the GMO in the state of export.
- Intended date or dates of the transboundary movement, if known.
- Taxonomic status, common name, point of collection or acquisition, and characteristics of recipient organism or parental organisms related to biosafety.

- Centres of origin and centres of genetic diversity, if known, of the recipient organism and/or the parental organisms and a description of the habitats where the organisms may persist or proliferate.
- Taxonomic status, common name, point of collection or acquisition, and characteristics of the donor organism or organisms related to biosafety.
- Description of the nucleic acid or the modification introduced, the technique used, and the resulting characteristics of the GMO.
- Intended use of the GMO or products thereof, namely, processed materials that are of GMO origin, containing detectable novel combinations of replicable genetic material obtained through techniques listed in Box 2.1.
- Quantity or volume of the GMO to be transferred.
- A previous and existing risk assessment report.
- Suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures, where appropriate.
- Regulatory status of the GMO within the state of export (for example, whether it is prohibited in the state of export, whether there are other restrictions, or whether it has been approved for general release) and, if the GMO is banned in the state of export, the reason or reasons for the ban.
- Result and purpose of any notification by the exporter to other states regarding the GMO to be transferred.
- A declaration that the above-mentioned information is factually correct.

ANNEX 11

SUMMARY OF INFORMATION RECOMMENDED TO BE COLLECTED AND COLLATED PRIOR TO THE COMMERCIAL RELEASE OF A GMO[1]

I. GENERAL INFORMATION

A. Name and address of the notifier (company or institute)

B. Name, qualifications and experience of the responsible scientist(s)

C. Title of the project

D. Designation and specification of the GMO and/or derived products

E. Where applicable, a detailed description of the method of production and manufacturing

F. Where appropriate, the conditions for placing on the market the food(s) or feed(s) produced from it, including specific conditions for use and handling

II. INFORMATION RELATING TO THE GMO

A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s):

» scientific name;

1 Adapted from: EU, 2001; EFSA, 2006a

- taxonomy (family genus, species, subspecies, cultivar);
- other names (usual name, strain name, etc.);
- phenotypic and genetic markers;
- degree of relatedness between donor and recipient or between parental organisms;
- description of identification and detection techniques;
- sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;
- description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts;
- organisms with which transfer of genetic material is known to occur under natural conditions;
- verification of the genetic stability of the organisms and factors affecting it;
- pathological, ecological and physiological traits:
  - classification of hazard according to the existing European Union's rules concerning the protection of human health and/or the environment;
  - generation time in natural ecosystems, sexual and asexual reproductive cycle; specific factors affecting reproduction, if any
  - information on survival, including seasonability and the ability to form survival structures;
  - pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism; possible activation of latent viruses (proviruses); ability to colonize other organisms;
  - antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;
  - involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.
- Sexual compatibility with other cultivated or wild species;

- Other potential interactions of the GMO with organisms in the ecosystem where it is usually grown, or elsewhere;
- Dissemination:
  - ways and extent (for example, an estimation of how viable pollen and/or seeds decline with distance) of dissemination;
  - specific factors affecting dissemination, if any.
- Nature of indigenous vectors:
  - sequence;
  - frequency of mobilization;
  - specificity;
  - presence of genes which confer resistance.
- History of previous genetic modifications.

B. Characteristics of the vector

- nature and source of the vector;
- sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO;
- frequency of mobilization of inserted vector and/or genetic transfer capabilities and methods of determination;
- information on the degree to which the vector is limited to the DNA required to perform the intended function.

C. Characteristics of the modified organism

- Information relating to the genetic modification:
  - methods used for the modification;
  - methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;
  - description of the insert and/or vector construction;
  - purity of the insert from any unknown sequence and information on the

degree to which the inserted sequence is limited to the DNA required to perform the intended function;

- methods and criteria used for selection;
- sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question, with particular reference to any known harmful sequence;
- location(s) of the insert(s) in the cells (integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination;
- in case of deletion, size and function of the deleted region(s).

- Information on the final GMO:
  - description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;
  - structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;
  - stability of the organism in terms of genetic traits;
  - rate and level of expression of the new genetic material; method and sensitivity of measurement;
  - parts of the organism where the insert is expressed (for example roots, stem, pollen, etc.);
  - activity of the expressed protein(s);
  - description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector;
  - sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;
  - information on how the genetically modified plant differs from the recipient plant in:
  - mode(s) and/or rate of reproduction;

- dissemination;
- survivability.
- history of previous releases or uses of the GMO;
- considerations for human health and animal health, as well as plant health:
- toxic or allergenic effects of the GMOs and/or their metabolic products;
- comparison of the modified organism with the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
- capacity for colonization;
- if the organism is pathogenic to humans who are immunocompetent:
- diseases caused and mechanism of pathogenicity, including invasiveness and virulence
- communicability
- infective dose
- host range, possibility of alteration,
- possibility of survival outside of human host
- presence of vectors or means of dissemination
- biological stability
- antibiotic resistance patterns,
- allergenicity,
- availability of appropriate therapies.

» (v) other product hazards.

III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT

A. Information on the release

» description of the proposed deliberate release, including the purpose(s) and foreseen products;

» foreseen dates of the release and time planning of the experiment, including frequency and duration of releases;

- methods for preparing and managing the release site, prior to, during and post-release, including cultivation practices and harvesting methods;
- size of the site;
- method(s) to be used for the release;
- quantities of GMOs to be released;
- disturbance on the site (type and method of cultivation, mining, irrigation, or other activities);
- worker protection measures taken during the release;
- post-release treatment of the site;
- techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment;
- information on, and results of, previous releases of the GMOs, especially at different scales and in different ecosystems.

B. Information on the environment (both on the site and in the wider environment):

- geographical location and grid reference of the site(s) (in case of notifications under part C the site(s) of release will be the foreseen areas of use of the product);
- physical or biological proximity to humans and other significant biota;
- proximity to significant biotopes, protected areas, or drinking water supplies;
- climatic characteristics of the region(s) likely to be affected;
- geographical, geological and pedological characteristics;
- flora and fauna, including crops, livestock and migratory species;
- description of target and non-target ecosystems likely to be affected;
- a comparison of the natural habitat of the recipient organism with the proposed site(s) of release;
- any known planned developments or changes in land use in the region which could influence the environmental impact of the release;
- presence of sexually compatible wild relatives or cultivated species.

IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT

A. Characteristics affecting survival, multiplication and dissemination

- biological features which affect survival, multiplication and dispersal;
- known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.);
- sensitivity to specific agents.

B. Interactions with the environment

- predicted habitat of the GMOs;
- studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses;
- genetic transfer capability
  - post-release transfer of genetic material from GMOs into organisms in affected ecosystems;
  - post-release transfer of genetic material from indigenous organisms to the GMOs;
- likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism;
- measures employed to ensure and to verify genetic stability; description of genetic traits which may prevent or minimize dispersal of genetic material; methods to verify genetic stability;
- routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.;
- description of ecosystems into which the GMOs could be disseminated;
- potential for excessive population increase in the environment;

- competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s);
- identification and description of the target organisms, if applicable;
- anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable;
- identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction;
- likelihood of post-release shifts in biological interactions or in host range;
- known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens;
- known or predicted involvement in biogeochemical processes and other effects on the abiotic environment;
- other potential interactions with the environment.

V. INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

A. Monitoring techniques

- methods for tracing the GMOs, and for monitoring their effects;
- specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques;
- techniques for detecting transfer of the donated genetic material to other organisms;
- duration and frequency of the monitoring.

B. Control of the release

- methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of release or the designated area for use;

- methods and procedures to protect the site from intrusion by unauthorized individuals;
- methods and procedures to prevent other organisms from entering the site;
- description of methods for post-release treatment of the site.

C. Waste treatment

- type of waste generated;
- expected amount of waste;
- description of treatment envisaged.

D. Emergency response plans

- methods and procedures for controlling the GMOs in case of unexpected spread;
- methods for decontamination of the areas affected, for example eradication of the GMOs;
- methods for disposal or sanitation of plants, animals, soil, etc. that were exposed during or after the spread;
- methods for the isolation of the area affected by the spread;
- plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

REFERENCES

Adair, D. & Irwin, R. 2008. A practical guide to containment. Plant biosafety in research greenhouses. *Information Systems for Biotechnology*. Virginia, USA, Blacksburg. Available at http://www.isb.vt.edu/cfdocs/greenhouse_manual.cfm

APHIS (Animal and Plant Health Inspection Service). 2008. Biotechnology: Compliance and the inspection process. Available at http://www.aphis.usda.gov/biotechnology/brs_main.shtml

Bartsch, D. 2005. Issues and challenges in monitoring GM crop-specific traits. *In* FAO, 2005.

Committee on the Biological Confinement of Genetically Engineered Organisms. 2004. *Biological confinement of genetically engineered organisms.* Washington DC, The National Academies Press.

Conner, A.J., Glare, T.R. & Nap, J.P. 2003. The release of genetically modified crops into the environment. Part II: Overview of ecological risk assessment. *The Plant Journal* 33: 19-46. Available at http://www.inai.org.ar/ogm/The%20relese.part%202.pdf

Convention on Biological Diversity (CBD). 2000. The Cartagena Protocol on Biosafety. Available at http://www.cbd.int/biosafety/about.shtml

Corich, V., Giacomini, A., Vendramin, E., Vian, P., Carlot, M., Concheri, G., Polone, E., Casella, S., Nuti, M.P. & Squartini, A. 2007. Long term evaluation of field-released genetically modified rhizobia. *Environ. Biosafety Res*. 6(3): 167-81.

CropLife International. 2005. Compliance management of confined field trials. CropLife International aisbl, Brussels. Available at http://www.croplife.org/librarypublications.aspx?wt.ti=Publications

Department of Biotechnology. 2006. Procedures, protocols and guidelines for regulation of genetically modified organisms. Ministry of Science and Technology, Government of India.

EU (European Union). 1990. Council Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms. OJ No L 117 of 8. 5. 1990, p.1. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31990L0219:EN:HTML

EU (European Union). 1998. Council Directive 98/81/EC of 26 October 1998 amending Directive 90/219/EEC, on the contained use of genetically modified micro-organisms. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31998L0081:EN:HTML

EU (European Union). 2001. 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. *OJ L 106, 17.4.2001, p. 1–39. Available at* http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:106:0001:0038:EN:PDF

EU (European Union). 2002a. Commission decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32001L0018:EN:HTML

EU (European Union). 2002b. Council Decision 2002/811/EC of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *OJ L 280, 18.10.2002, p. 27–36. Available at* http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:280:0027:0036:EN:PDF

EU (European Union). 2003a. Regulation No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on transboundary movements of genetically modified organisms. *OJ L 287, 5.11.2003, p. 1–10. Available at* http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:287:0001:0010:EN:PDF

EU (European Union). 2003b. Regulation No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. *OJ L 268, 18.10.2003, p. 24–28*. Available at http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0024:0028:EN:PDF

EU (European Union). 2004. Commission Regulation No 65/2004 of 14 January 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms. *OJ L 10, 16.1.2004, p. 5–10. Available at* http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:010:0005:0010:EN:PDF

EFSA (European Food Safety Authority). 2006a. Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO). *EFSA Journal* 99: 1-100. Available at http://www.efsa.europa.eu/en/scdocs/scdoc/99.htm

EFSA (European Food Safety Authority). 2006b. Guidance document for the risk assessment of genetically modified micro-organisms and their derived products intended for food and feed use by the Scientific Panel on Genetically Modified Organisms (GMO). *EFSA Journal* 374: 1-115. Available at http://www.efsa.europa.eu/en/scdocs/scdoc/374.htm

EPA (U.S. Environmental Protection Agency). 2008. Introduction to biotechnology regulation for pesticides. Available at http://epa.gov/pesticides/biopesticides/regtools/biotech-reg-prod.htm

FAO. 1995. *Code of Conduct for responsible fisheries*. Rome.

FAO. 2001. *Glossary of biotechnology for food and agriculture*. Rome. Available at http://www.fao.org/biotech/index_glossary.asp

FAO. 2005. Genetically Modified Organisms in crop production and their effects on the environment: methodologies for monitoring and the way ahead. Rome. Available at http://www.fao.org/docrep/009/a0802e/a0802e00.HTM

Ghosh, P K. 2002. Indian legal framework and scientific progress. *Proceedings of Asia Regional Workshop on risk assessment and risk management in implementing the Cartagena Protocol on Biosafety*, pp. 13-28.

Halsey, M.E. 2006. *Integrated confinement system for genetically engineered plants*. Program for Biosafety Systems. St Louis, Missouri, USA, Donald Danforth Plant Science Center. Available at http://programs.ifpri.org/pbs/pbspubs.asp

Heinemann, J.A. & Traavik, T. 2004. Problems in monitoring horizontal gene transfer in field trials of transgenic plants. *Nature Biotechnology* 23(4): 488.

Hu, W., Wang, Y., & Zhu, Z. 2007. Progress in the evaluation of transgenic fish for possible ecological risk and its containment strategies. *Sci China C Life Sci*. 50(5): 573-579.

Jansson, J.K., Bailey, M. & van Elsas, J.D. (eds). 2000. *Tracking Genetically Modified Micro-organisms (Biotechnology Intelligence Unit 2)*. Austin, Texas, USA, R.G. Landes Company. Further information available at http://ec.europa.eu/research/quality-of-life/gmo/06-tools/06-07-project.html

Jepson, P. 2005. Challenges to the design and implementation of effective monitoring for GM crop impacts: Lessons from conventional agriculture. *In* FAO, 2005.

Layton, R. 2005. Monitoring strategies and management of GM crops: An industry perspective. *In* FAO, 2005.

NIH (National Institutes of Health). 2009. NIH Guidelines for research involving recombinant DNA molecules. Bethesda, Maryland, USA, Department of Health and Human Services, National Institutes of Health. Available at http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

OECD (Organization for Economic Co-operation and Development). 1997-2009. Consensus Documents for the Work on Harmonisation of Regulatory Oversight in Biotechnology. Available at http://www.oecd.org/document/51/0,2340,en_2649_34387_1889395_1_1_1_1,00.html

Sahai, S. 2005. Assessing the socio-economic impact of GE crops. *In* FAO, 2005.

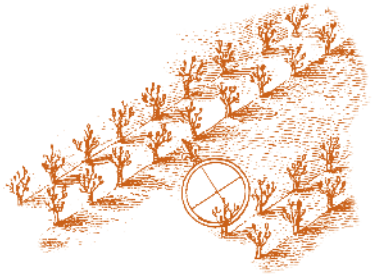
Sonnino, A., Dhlamini, Z., Santucci, F.M. & Warren, P. 2009. Socio-economic impacts of non-transgenic biotechnologies in developing countries: the case of plant micropropagation in Africa. FAO, Rome, Italy.

WHO (World Health Organization). 2004. Laboratory Biosafety Manual, 3rd Edition. Geneva. Available at http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Wilhelm, R., Beissner, L. & Schiemann, J. 2003. Concept for the realization of a GMO monitoring in Germany. Nachrichtenblatt des deutschen Pflanzenschutzdienstes 55. Available at http://www.biosicherheit.de/pdf/dokumente/bba_monitoring.pdf

Zbikowska, H.M. 2003. Fish can be first – advances in fish transgenesis for commercial applications. *Transgenic Research* 12: 379–389.

Zühart, W., Benzler, A., Berhorn, F., Sukopp, U. & Graef, F. 2008. Determining indicators, methods and sites for monitoring potential adverse effects of genetically modified plants to the environment : the legal and conceptional framework for implementation. *Euphytica* 164: 845-852.



Design and layout: Pietro Bartoleschi and Arianna Guida (Studio Bartoleschi)
Cover illustrations elaborated from “l’Encyclopédie Diderot et d’Alembert”
Printed in Italy on ecological paper, Forest Stewardship Council (FSC) certified, May 2011

Biosafety Resource Book

MODULE D

TEST AND POST-RELEASE MONITORING OF GMOs

addresses the use and monitoring of GMOs under containment, confinement and limited field trials, as well as the monitoring of commercially released GMOs. It also covers surveillance and emergency planning.

For additional information please consult
www.fao.org/biotech
or contact
biotech-admin@fao.org

MODULE

Biosafety Resource Book

e

LEGAL ASPECTS

●●●●○

FAO FIAT PANIS

MODULE

Biosafety Resource Book

LEGAL ASPECTS

Anna Milena Zivian
Alessandra Sensi
Carmen Bullón Caro

Food and Agriculture
Organization of
the United Nations
Rome, 2011

LIST OF CONTRIBUTORS

This module has been prepared based on the lectures and contributions of:

Carmen Bullón Caro
Development Law Service
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy

Patricia Jere
Ministry of Justice
Lusaka, Zambia

Patricia Kameri-Mbote
Faculty of Law
University of Nairobi
Nairobi, Kenya

Bala Ravi Sekhara Pillai
M.S. Swaminathan Foundation
Chennai, India

Alejandra Sarquis
Secretaría Técnico-Administrativa
Consejo Agropecuario del Sur (CAS)
Instituto Interamericano de Cooperación
para la Agricultura (IICA)
Santiago, Chile

Alessandra Sensi
Research and Extension Branch
Food and Agriculture Organization of
the United Nations (FAO)
Rome, Italy
(present address:
EuropeAid Co-operation Office, Unit A.3
Centralised Operations for Europe,
the Mediterranean and the Middle East
Brussels, Belgium)

Carmen Vicién
Facultad de Agronomía
Universitad of Buenos Aires
Buenos Aires, Argentina

Anna Milena Zivian
Environmental Studies
University of California, Santa Cruz
Santa Cruz, California, USA
(present address:
Ocean Conservancy,
Washington, DC, USA)

CONTENTS

MODULE e

LIST OF ABBREVIATIONS

ABS	Access and benefit-sharing
AIA	Advanced Informed Agreement
ASEAN	Association of Southeast Asian Nations
BCH	Biosafety Clearing-House
CBD	Convention on Biological Diversity
Codex	Codex Alimentarius
COP-MOP	Conference of the Parties serving as the meeting of the Parties to the Protocol
CPB	Cartagena Protocol on Biosafety
CPM	Commission on Phytosanitary Measures
DNA	Deoxyribonucleic acid
EC	European Commission
EIA	Environmental Impact Assessment
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FFP	Food, or feed or for processing
GATT	General Agreement on Tariffs and Trade
GDP	Good Development Principles
GMO	Genetically modified organism
IP	Identity preservation
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
IUCN	International Union for Conservation of Nature
LMO	Living modified organism
NGO	Non-governmental organization
OECD	Organisation for Economic Co-operation and Development
OIE	Office International des Epizooties
PGRFA	Plant Genetic Resources for Food and Agriculture
PRA	Pest Risk Analysis
SPM	Sanitary and Phytosanitary Measures
SPS	Sanitary and Phytosanitary Agreement
TBT	Technical Barriers to Trade
TRIPS	Agreement on Trade-related Aspects of Intellectual Property Rights
UN	United Nations
UNECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
UNIDO	United Nations Industrial Development Organization
UPOV	International Union for the Protection of New Varieties of Plants
USDA	United States Department of Agriculture
WHO	World Health Organization
WTO	World Trade Organization

CHAPTER 1

INTRODUCTION: OVERVIEW OF EXISTING LEGAL FRAMEWORKS ON BIOTECHNOLOGY AND BIOSAFETY

Legal provisions to regulate biotechnology and biosafety issues exist at every level of government. This includes transnational (e.g. the United Nations [UN]), regional (such as the European Union [EU] or the African Union [AU]), national, and subnational levels.

Biosafety is defined as a "Set of measures or actions addressing the safety aspects related to the application of biotechnologies and to the release into the environment of transgenic plants and organisms, particularly microorganisms, that could negatively affect plant genetic resources, plant, animal or human health, or the environment" (UNEP Glossary, 2007).

The term "**biosafety**" is generally used to describe frameworks of policy, regulation and management to control potential risks associated with the use of new biotechnologies ("New biotechnologies" being a term used to differentiate processes that use modern techniques of biotechnology, such as recombinant DNA techniques, from traditional breeding and improvement techniques used in agriculture), including their use, release and transboundary movements. Biosafety frameworks may also address risk communication and other issues such as potential positive or negative socio-economic impacts. Many of the legal instruments addressing biosafety have primary goals, such as the preservation of biodiversity, consumer protection, public participation and information, development and trade, and address biosafety only indirectly.

BIOSAFETY
Generally used to describe frameworks of policy, regulation, and management to control potential risks associated with the use of new biotechnologies.

BOX 1.1

BIOSAFETY AND AGRICULTURAL BIOTECHNOLOGY INSTRUMENTS (GLOWKA, 2003)

BIOSAFETY AND AGRICULTURAL BIOTECHNOLOGY INSTRUMENTS
A classification of instruments addressing agricultural biotechnology and biosafety into three different areas: biosafety instruments, food safety instruments, and consumer protection instruments.

Glowka (2003) proposes a classification of instruments addressing agricultural biotechnology and biosafety into three different areas: biosafety instruments, food safety instruments, and consumer protection instruments.

Biosafety instruments represent the primary source of law on modern biotechnology in the world today. Biosafety instruments address the risks posed to the environment and human health when GMOs are released into the environment either for research (e.g. small-scale or field-testing) or for commercial purposes. Biosafety instruments also address contained use of GMOs.

Food safety instruments address the risks posed to humans by genetically modified foods. The general goal of these instruments is to minimize risks to humans presented by GMOs or their products used as foods themselves or as ingredients in food. Ideally the entire human food chain is examined, moving from the farm to the kitchen table. A related area is animal feed safety.

Consumer protection instruments address a range of issues primarily in that area of biotechnology related to food or feed products. The labelling of end products resulting from genetic engineering, such as food or animal feed, is the primary area addressed. In general, these instruments are designed to (1) protect the consumers' right to know and the right to make informed choices and (2) ensure fair trade practices to ensure that consumers are not victimized by false or misleading claims about a product.

Legal frameworks on biosafety include binding and non-binding international and regional agreements and national laws, regulations and guidelines. This chapter explains the different levels, types, and purposes of these instruments and how they may interrelate. Chapter 2 of this module explains specific international instruments, and Chapter 3 discusses elements of different legal frameworks and biosafety instruments and how they are transposed into national biosafety frameworks.

LEGAL FRAMEWORKS ON BIOSAFETY
Include binding and non-binding international and regional agreements and national laws, regulations and guidelines, dealing with the regulation of biotechnology and biosafety.

International instruments to regulate biotechnology and biosafety include treaties, conventions, and agreements that have been agreed upon by several nations. A number of existing agreements have been launched and are implemented by UN agencies, although not all its Members are signatories or parties to all these agreements. In addition, the World Trade Organization (WTO), with its 153 Members[1], plays a large role in determining how biotechnology is regulated at the national level.

Among regional instruments, the EU regulatory framework is one of the most extensive, covering issues including import, cultivation, monitoring and labelling of GMOs or GMO-derived material. Some subnational instruments may also have a role in this framework.

International and regional instruments provide guidance and general principles that are then adopted into national legislation and regulatory policy and applied at the national level. Different countries may choose different means of implementing internationally agreed principles, through both binding and non-binding national instruments.

INTERNATIONAL AND REGIONAL INSTRUMENTS
Provide guidance and general principles that are then adopted into national legislation and regulatory policy and applied at the national level.

In some national legal systems, international agreements may need to be ratified or transposed into national law by the signatories to be put into practice. This makes national frameworks particularly relevant for the implementation of international and regional agreements.

1 As of January 2010

States also enact their own biotechnology legislation. There is a wide range of solutions that may be adopted at national level, including a variety of schemes, frameworks and instruments for addressing biosafety and other issues related to biotechnology, such as liability and redress and coexistence among genetically modified, conventional and organic crops. In addition, legislation not expressly directed at regulating biotechnology may nonetheless apply to specific areas, including living modified organisms (LMOs) or genetically modified organisms (GMOs). Trade issues intervene as well, with questions of whether GMO regulation may affect free markets among signatories to trade agreements.

BINDING AND NON-BINDING INSTRUMENTS
Instruments that either entail an obligation under international law or do not have any binding force, also referred to as hard law and soft law.

This plethora of legal instruments operating at different levels may create confusion and, on occasion, overlaps and conflicts. It is therefore important to understand the range of options for national biosafety legislation and the current status and context for addressing biosafety issues.

1.1 TYPES OF INSTRUMENTS USED TO REGULATE BIOTECHNOLOGY

International instruments include several different types of treaties and agreements addressing – directly or only indirectly – biotechnology and biosafety. These instruments comprise both **binding** (i.e., entailing an obligation under international law) and **non-binding instruments** ("hard" and "soft" law).

TREATY
An international agreement concluded between States in written form and governed by international law, whether embodied in a single instrument or in two or more related instruments and whatever its particular designation.

The Vienna Convention on the Law on Treaties (1969), defines a **treaty** as: "*an international agreement concluded between States in written form and governed by international law, whether embodied in a single instrument or in two or more related instruments and whatever its particular designation*" (article 2[1][a]). Key to this definition is that a treaty is an *international* agreement and that it is governed by *international law*.

BOX 1.2

DEFINITIONS OF HARD AND SOFT LAW (UNEP GLOSSARY, 2007)

Hard law
Term used to describe the legally binding nature of various agreements or provisions, which leave no or little room for discretion.

Soft law
The term used for quasi-legal instruments which do not have any binding force, or those whose binding force is somewhat "weaker" than the binding nature of traditional law, often referred to as "hard law". In the international context, soft law consists of non-treaty obligations which are therefore non-enforceable and may include certain types of declarations, guidelines, communications and resolutions of international bodies.

BOX 1.3

DEFINITIONS: ACCESSION, RATIFICATION, AND IMPLEMENTATION (UNEP GLOSSARY, 2007)

Accession: Act whereby a state becomes a party to an international agreement already negotiated and closed for signature. Accession has the same legal effect as ratification, although an acceding state has not signed the agreement.

Ratification: Formal process by which a head of state or appropriate government official or authority signs a document which signals the consent of the state to become a party to an international agreement once the agreement has entered into force and to be bound by its provisions.

Implementation: For a party to an international agreement, [the] process of adopting relevant policies, laws and regulations, and undertaking necessary actions to meet its obligations under the agreement.

DEFINITIONS: ACCESSION, RATIFICATION, AND IMPLEMENTATION
Provides definitions of the different processes of how a state can deal with international agreements.

This means that parties signing the agreement cannot unilaterally interpret it, and agree to be governed by international law – the presiding authority is not the nation, but the governing body or system created by the treaty in question and the rules of interpretation are not any national legal system but the principles commonly agreed by the treaty and the principles of international law.

BINDING INSTRUMENTS carry the force of law and require signatories to comply with the agreements as adopted.

Binding instruments (hard law) carry the force of law and require signatories to comply with the agreements as adopted (as discussed earlier, this may include ratification and/or transposition of agreements into national frameworks through implementing legislation). Some binding agreements introduce mechanisms for dispute resolution.

NON-BINDING AGREEMENTS are normally the result of processes that involve consensus building among countries; hence, their "moral authority" is a result of the legitimacy of this consensus.

Non-binding agreements (soft law) include codes of conduct, guidelines, manuals on "best practices", recommendations, declarations of principle, and action programmes. As opposed to binding agreements, these do not create binding obligations and are not legal instruments enforceable by the national institutions. Consequently, there is no formal need for ratification or transposition into national legislation and no means of compulsory compliance. Non-binding agreements offer the advantage of being faster and simpler to adopt than binding agreements, and provide more flexible means for update and amendment.

Non-binding agreements are normally the result of processes that involve consensus building among countries; hence, their "moral authority" is a result of the legitimacy of this consensus. They are often implemented as "de facto" legislation and can later become or be incorporated into binding agreements (Hannam and Boer, 2002). Creation under the auspices of internationally recognized organizations (such as UN organizations); legitimacy through participation in framing and drafting by representatives of a broad range of international and national authorities; and adoption by a majority of international actors (especially states) can create both practical and moral incentives to comply.

Table 1.1 | Definitions and examples of international instruments

Instrument	Definition	Binding or non-binding	Example	Goals – from selected examples
Code of conduct	Set of rules to guide behaviour and decisions	Non-binding	FAO Code of Conduct on Responsible Fisheries	Establish principles, serve as reference, provide guidelines, provide standards of conduct, etc.
Guidelines	Statement, indication of procedure; guidance for decisions	Non-binding	UNEP Technical Guidelines on Biosafety http://www.unep.org/biosafety/Documents/Techguidelines.pdf	Help achieve "international information exchange, cooperation, harmonization, and agreement"
Best practices	Benchmarks using techniques considered to be the most effective/efficient	Non-binding	OECD Best Practice Guidelines for Biological Resource Centres http://www.oecd.org/dataoecd/7/13/38777417.pdf	A target and guidelines for managing and improving the quality of biological resource centres that store and supply biological materials and information
Recommendations	Formal expression of an advisory nature of the will of the governing body of an international organization or international agreement.	Non-binding	European Commission Recommendation 2004/787/EC of 4 October 2004 on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:348:0018:0026:EN:PDF	Facilitating a coordinated approach to adopting sampling and detection techniques
Declaration (of Principle)	A formal statement of aspirations issued by a meeting. Usually issued by high-level representatives.	Non-binding unless required by treaty	1992 Rio Declaration on Environment and Development	Principle 15 on precaution: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (http://www.unep.org/Documents.Multilingual/Default.asp?DocumentID=78&ArticleID=1163).

Instrument	Definition	Binding or non-binding	Example	Goals – from selected examples
Position Statement	A statement of goals related to a particular subject	Non-binding	United Kingdom Joint Nature Conservation Committee position statement on biotechnology	"We are solely concerned with potential impacts of GMO releases on the living environment and on sustainable use of our natural resources, including protected sites and the wider countryside. We have no locus on matters of public health and safety. The agencies, working through the JNCC, advocate using the precautionary principle where commercial releases are proposed…" (http://www.jncc.gov.uk/page-2992)
Programme of Action	Guidance for designing and implementing policies to achieve joint goals, often as expressed in other agreements	Non-binding	UNEP Global Programme of Action for the Protection of the Marine Environment from Land-Based Activities http://www.gpa.unep.org/	"…preventing the degradation of the marine environment from land-based activities by facilitating the realization of the duty of States to preserve and protect the marine environment. It is designed to assist States in taking actions individually or jointly within their respective policies, priorities and resources, which will lead to the prevention, reduction, control and/or elimination of the degradation of the marine environment, as well as to its recovery from the impacts of land-based activities" (GPA)
Treaty	International agreement concluded between states in written form and governed by international law, whether embodied in a single instrument or in two or more related instruments and whatever its particular designation (Vienna Convention on the Law of Treaties).	Binding	International Treaty on Plant Genetic Resources for Food and Agriculture http://www.planttreaty.org/	"No country is self-sufficient in plant genetic resources; all depend on genetic diversity in crops from other countries and regions. International cooperation and open exchange of genetic resources are therefore essential for food security. The fair sharing of benefits arising from the use of these resources has for the first time been practically implemented at the international level through the Treaty and its Standard Material Transfer Agreement" (www.planttreaty.org)

Instrument	Definition	Binding or non-binding	Example	Goals – from selected examples
Convention	A binding agreement between states. Generally used for formal multilateral instruments with a broad number of parties.	Binding	Convention on Biological Diversity www.cbd.int	"The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding" (Article 1, CBD, at http://www.cbd.int/convention/articles.shtml?a=cbd-01)
Protocol	(1) International legal instrument appended or closely related to another agreement, which constitutes a separate and additional agreement and which must be signed and ratified by the parties to the convention concerned. Protocols typically strengthen a convention by adding new, more detailed commitments. (2) Rules of diplomatic procedure, ceremony and etiquette. (3) Department within a government or organization that deals with relations with other missions.	Binding	Cartagena Protocol bch.cbd.int/protocol	In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements (Article 1, CPB, at http://www.cbd.int/biosafety/articles.shtml?a=cpb-01)

Instrument	Definition	Binding or non-binding	Example	Goals – from selected examples
Agreement	(1) Generic term for an international legally binding instrument. In this sense, encompasses several instruments, such as treaties, conventions, protocols or oral agreements. (2) Specific term used to designate international instruments that are sic "less formal", thus corresponding to soft law and deal with a narrower range of subject matter than treaties.	Binding	Agreement on Application of Sanitary and Phytosanitary Measures (SPS)	Members shall ensure that their sanitary and phytosanitary measures do not arbitrarily or unjustifiably discriminate between Members where identical or similar conditions prevail, including between their own territory and that of other Members. Sanitary and phytosanitary measures shall not be applied in a manner which would constitute a disguised restriction on international trade (Article 2, Section 3, SPS at http://www.wto.org/english/tratop_e/sps_e/spsagr_e.htm)

Where available, definitions are *adapted from*: UNEP - Glossary of Terms for Negotiators of Multilateral Environmental Agreements (2007).

Binding agreements include treaties, conventions and international agreements. Other terms used for "treaty" include "Compact, Solemn Declaration, Administrative Agreement, Protocol of Decisions, Platform, Concordat, Agreed Minute and Terms of Reference" (Aust, 2000).

One may differentiate between agreements that deal directly with biosafety, such as the Cartagena Protocol on Biosafety (CPB) (see section 2.2.2), and others that affect it indirectly, such as the WTO SPS (section 2.2.3) agreement, which do not mention biosafety directly, but nonetheless have a direct bearing on adoption of national biosafety frameworks. Some agreements may overlap, interrelate, or conflict, especially those on trade and those on biosafety.

Table 1.2 | **International agreements related to biosafety**
(see section 2 for additional discussion)

International agreements	Trade related	Non-trade related
Binding	Convention on Biological Diversity Cartagena Protocol on Biodiversity Agreement on Application of Sanitary and Phytosanitary Measures Agreement on Technical Barriers to Trade International Plant Protection Convention Law of the Sea Agreement on Trade-related Aspects of Intellectual Property Rights	Aarhus Convention The International Treaty on Plant Genetic Resources for Food and Agriculture
Non-binding	Codex Alimentarius International Union for Conservation of Nature position statement The Code of Conduct for the Import and Release of Exotic Biological Control Agents (1996)	Organization for Economic Co-operation and Development safety considerations Agenda 21 United Nations Industrial Development Organization Code of Conduct FAO Code of Conduct on Responsible Fisheries United Nations Environment Programme Technical Guidelines on Biosafety The UN Guidelines for Consumer Protection

INTERNATIONAL AGREEMENTS RELATED TO BIOSAFETY
A list of international instruments having a direct or indirect bearing on biosafety frameworks is provided.

Table 1.2 shows several agreements related to biosafety, including binding, non-binding and trade-related agreements. The relationships between these agreements will be discussed in section 2.5.

CHAPTER 2

INTERNATIONAL FRAMEWORKS ON BIOSAFETY

2.1 DESCRIPTION OF SELECTED LEGAL INSTRUMENTS ADDRESSING BIOSAFETY

DESCRIPTION OF SELECTED LEGAL INSTRUMENTS ADDRESSING BIOSAFETY
This section describes some of the most influential and widely applicable legal instruments addressing biosafety.

This section describes some of the most influential and widely applicable legal instruments addressing biosafety. Discussed first are binding instruments, followed by a discussion of non-binding instruments that nonetheless form an important part of international practice. Both categories include standard-setting instruments, which produce international standards and guidelines. A more inclusive list of instruments may be found in Annex 1.

2.2 INTERNATIONAL BINDING INSTRUMENTS ON BIOSAFETY

The following international agreements are binding upon their signatories and are highly relevant to biosafety and biotechnology. Most of them are directly aimed at regulating products of biotechnology; others do not explicitly mention biotechnology but have trade-related effects on biosafety decisions.

This section looks at eight important, binding international agreements. The Convention on Biological Diversity (CBD) and its Cartagena Protcol on Biosafety (CPB), The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS), the Agreement on Technical Barriers to Trade (TBT), and the WTO Agreement on Trade-related Aspects of Intellectual Property Rights (TRIPS) condition how governments regulate GMOs or LMOs. The Aarhus Convention includes specific provisions related to biosafety. The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) is not directly related to biosafety issues but interacts with the CBD. The International Plant Protection Convention (IPPC), the Office International des Epizooties (OIE) and the Codex Alimentarius (Codex) serve as a basis for standards some of which include provisions on biosafety.

INTERNATIONAL BINDING INSTRUMENTS ON BIOSAFETY
A discussion of eight important binding international agreements on biosafety is provided. Most of them are directly aimed at regulating products of biotechnology; others do not explicitly mention biotechnology but have trade-related effects on biosafety decisions.

2.2.1 The Convention on Biological Diversity (CBD) (1992)[1]

Definition: Biodiversity (UNEP Glossary, 2007)

Biodiversity
Shorthand for biological diversity. Variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.

CONVENTION ON BIOLOGICAL DIVERSITY (CBD)
The Convention establishes three main goals: the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of the benefits from the use of genetic resources.

The **Convention on Biological Diversity (CBD)** addresses biosafety in two articles: Article 8(g) and Article 19. Article 8(g) requires each contracting party domestically to regulate or manage the risks associated with the use and release of LMOs resulting from biotechnology likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, including risks related to alien invasive species. Risks to human health are also to be taken into account.

1 Entered into force 23 December 1993. As of January 2010, 193 Parties (168 Signatures).

The principles of prevention and precaution apply to the use and release of LMOs. The distinction between LMO and GMO arises because the CBD does not apply to processed food containing or derived from GMOs, but only to GMOs that are intended to be used directly as agricultural inputs, food, feed, or for processing (FFP).

Contracting parties undertake to introduce appropriate procedures to require impact assessment of proposed projects likely to have significant adverse effects on biodiversity (Art. 14[1][a]). The objective is to avoid or minimize such effects. Public participation in the procedures should be allowed where appropriate. Other relevant obligations include those on reciprocity, notification, exchange of information with other states and international organizations where activities in one party or state may adversely affect the biodiversity of another party or an area beyond the limits of any national jurisdiction (Art. 14[1][c, d]). Parties are to create emergency response arrangements at the national level and joint contingency plans with other states (Art. 14[1][e]). Parties are under obligation to transfer environmentally sound technology (including biotechnology) relevant to the conservation and sustainable use of biodiversity (Art. 16[1]).

BOX 2.1

ENVIRONMENTAL IMPACT ASSESSMENT (EIA)

Environmental Impact Assessment

Process by which the environmental consequences of a proposed project or programme are evaluated and alternatives are analysed.

EIA is an integral part of the planning and decision-making processes.

Article 19 refers to "Handling of biodiversity and distribution of its benefits." The first two sections obligate signatories to ensure that source countries for genetic material also share in biotechnological research and benefits based on the genetic resources they provide. Article 19(3) anticipates a protocol to the CBD "setting out appropriate procedures, including, in particular, advance informed agreement, in the field of the safe transfer, handling and use of any living modified organism resulting from biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity", which resulted in the adoption in 2000 of the CPB (discussed below in section 2.2.2). Article 19(4) of the CBD creates a bilateral obligation for a state party to provide information on an LMO prior to providing it to another party. This information includes any available information on the regulatory measures taken by the exporting party and any available information on the potential adverse impact of a particular LMO.

2.2.2 The Cartagena Protocol on Biosafety to the Convention on Biological Diversity (2000)[2]

THE CARTAGENA PROTOCOL ON BIOSAFETY
The objective of the Protocol is to contribute to ensuring adequate levels of protection in the field of safe transfer, handling and use of LMOs that may have adverse effects on the conservation and sustainable use of biodiversity taking into account risks to human health focusing in particular on transboundary movements.

The objective of the Protocol is to contribute to ensuring adequate levels of protection in the field of safe transfer, handling and use of LMOs that may have adverse effects on the conservation and sustainable use of biodiversity, taking into account risks to human health, focusing in particular on transboundary movements (Art. 1).

The Protocol specifically applies to transboundary movement, transit, handling and use of LMOs that may have adverse effects on biodiversity conservation and sustainable use, taking into account risks to human health (Art. 4). The Protocol applies only to the movement of LMOs between contracting parties. There is only one exception to the scope of the Protocol: it does not apply to the transboundary movement of LMOs that are pharmaceuticals for human use that are addressed by other relevant international agreements or organizations (Art. 5).

2 Entered into force 23 December 1993. As of January 2010, 193 Parties (168 Signatures).

In general, each party is obligated to take the necessary and appropriate legal, administrative and other measures to implement the Protocol's obligations and to ensure that the development, handling, transport, use, transfer and release of LMOs are undertaken in a manner that prevents or reduces risks to biodiversity, taking into account any risk to human health (Art. 2). Each party can take more protective action to conserve and sustainably use biodiversity, provided the action is consistent with the Protocol (Art. 2[4]).

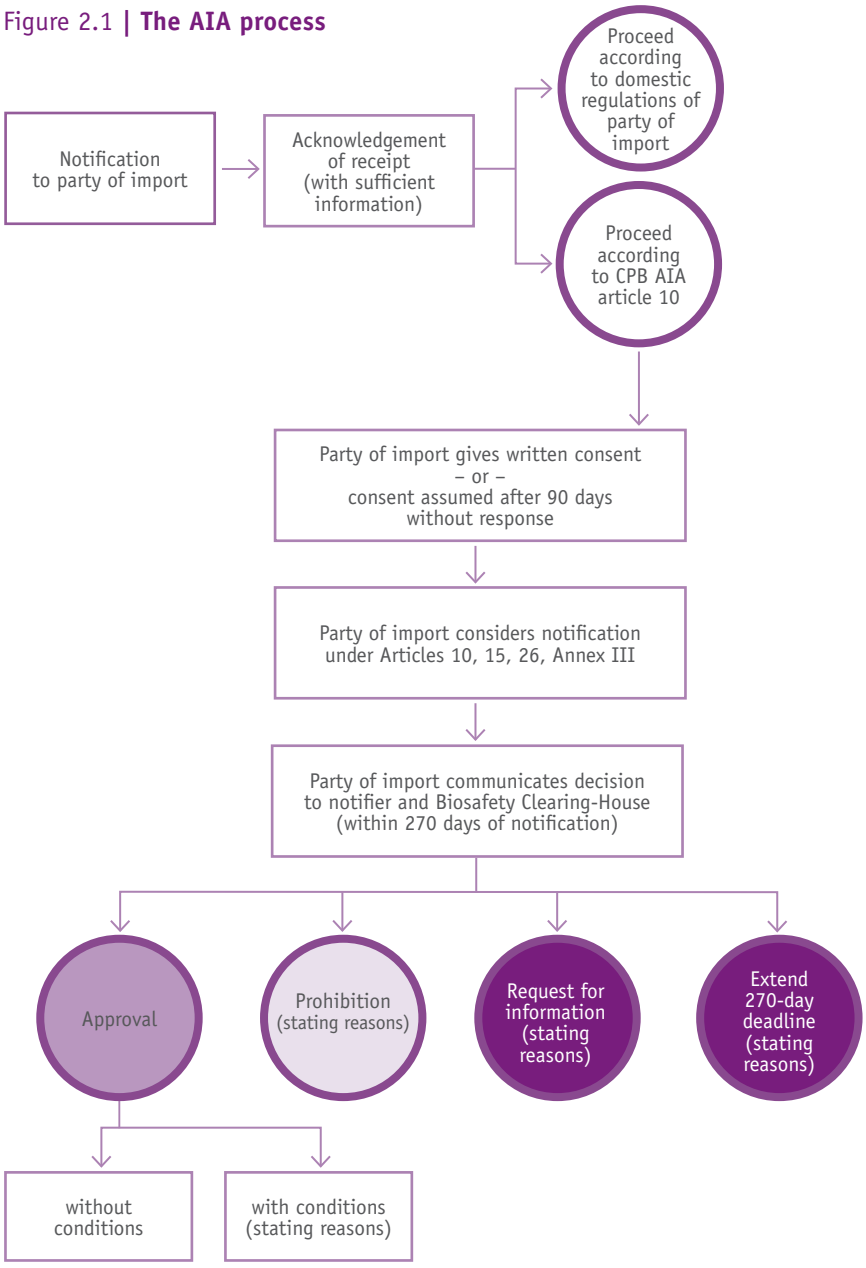
ADVANCED INFORMED AGREEMENT (AIA)
Describes the process for notification and subsequent approval of a first-time import of LMOs intended for introduction into the environment in order to avoid potential adverse effects on the conservation and sustainable use of biodiversity in the receiving environment.

The Biosafety Protocol focuses on the evaluation of and notification between the state parties for LMOs destined for export and subsequent import. It sets out an **Advanced Informed Agreement (AIA)** describing the process for notification and subsequent approval of a first-time import of LMOs intended for introduction into the environment in order to avoid potential adverse effects on the conservation and sustainable use of biodiversity in the receiving environment (Art. 7[10, 12]).

The AIA procedure requires, prior to the first intentional introduction into the environment of the importing party: (a) the notification of the party of export containing certain information, (b) the acknowledgment of its receipt, and (c) the written consent of the importing party (see Figure 2.1) (Art. 8, Art. 9). Criteria are provided for decision-making on importation (Art. 10). Most notably, decisions of the contracting party of import must be made according to a risk assessment (Art. 15).

There are four categories of exceptions to the AIA procedure – LMOs in transit (Art. 6[1]); LMOs for contained use (Art. 6(2); LMOs identified in a decision of the Conference of Parties/Meeting of Parties (COP-MOP) as not likely to have adverse effects on biodiversity conservation and sustainable use (Art. 7[4]); and LMOs intended for direct use as food, feed or for processing (Art. 11).

Figure 2.1 | **The AIA process**



Adapted from: Mackenzie *et al.*, 2003.

BIOSAFETY CLEARING-HOUSE (BCH)
Created under the Protocol, to (a) facilitate information exchange and (b) assist parties in implementing the Protocol, with particular attention to developing countries and countries that are centres of origin and of genetic diversity.

For LMOs intended for direct use as food or feed, or for processing, the contracting party that makes a final decision for domestic use must notify the **Biosafety Clearing-House (BCH)** (Art. 11).

The BCH was established to (a) facilitate information exchange and (b) assist parties in implementing the Protocol, with particular attention to developing countries and countries that are centres of origin and of genetic diversity (Art. 20[1]).

The exemption for AIA does not apply to decisions on field trials. Even though AIA does not apply, a contracting party may still take an import decision under its domestic regulatory framework, provided this is consistent with the Protocol (Art. 11[4]).

When it lacks a domestic regulatory framework, a developing country contracting party, or a party with a transition economy, can declare through the BCH that its decision on the first import of an LMO for direct use as food, feed or for processing will be pursuant to a risk assessment (Art. 11[6]). Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of potential adverse effects should not prevent the contracting party of import from taking a decision, as appropriate, in order to avoid or minimize potential adverse effects (Art. 11[8], Art.10[6]).

BOX 2.2

DEFINITIONS OF RISK ASSESSMENT AND RISK MANAGEMENT

Risk assessment:
The evaluation of the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing Member according to the sanitary or phytosanitary measures which might be applied, and of the

associated potential biological and economic consequences; or the evaluation of the potential for adverse effects on human or animal health arising from the presence of additives, contaminants, toxins or disease-causing organisms in food, beverages or feedstuffs.

From: WTO SPS, Annex A: Definitions, available at http://www.wto.org/english/docs_e/legal_e/15sps_02_e.htm#annA

Risk assessment:
The [risk assessment] methodology described in Annex III of the Protocol follows the conventional risk assessment paradigm, beginning with identification of a potential hazard, such as characteristics of an LMO, which may have an adverse effect on biodiversity. Risks are then characterized based on combined evaluation of the likelihood of adverse effects, and the consequences should those effects be realized.

From CBD discussion of risk assessment, available at http://www.cbd.int/biosafety/issues/risk.shtml

Risk management is the second step in conventional risk assessment, and incorporates the information gained during the risk assessment phase in order to make appropriate decisions on how to manage any risks that may exist. It is a key element in the conventional risk analysis paradigm, and is discussed in several international agreements (see selections below).

...establish and maintain appropriate mechanisms, measures and strategies to regulate, manage and control risks identified in the risk assessment...

From CBD Article 16, Risk Management

Risk management measures for foods derived from modern biotechnology should be proportional to the risk, based on the outcome of the risk assessment and, where relevant, taking into account other legitimate factors....

From Codex Principles for the risk analysis of foods derived from modern biotechnology

Pest risk management (for quarantine pests) is the "Evaluation and selection of options to reduce the risk of introduction and spread of a pest."

Adapted from: ISPM 11, 2004.

RISK ASSESSMENT AND RISK MANAGEMENT
Short definitions of these processes are provided. For details, please refer to Module 3: Risk analysis

Risk assessment and risk management are key requirements in the CPB for decisions on whether to allow the import of an LMO. The risk assessment must be consistent with criteria enumerated in Annex III (Art. 15). The Protocol also specifies general risk management measures and criteria. Risk analysis procedures are discussed further in section 3.6 of this manual.

Under Article 26, the contracting parties reaching import decisions under the Protocol or under domestic legal measures implementing the Protocol may account for socio-economic considerations arising from the impact of LMOs on biodiversity conservation and sustainable use, especially with regard to the value of biodiversity to indigenous and local communities. The parties are encouraged to cooperate on research and information exchange on any socio-economic impacts of LMOs, especially on indigenous and local communities (Art. 26[2]).

SUSTAINABLE USE
Use in a way and at a rate that does not lead to the long-term degradation of the environment, thereby maintaining its potential to meet the needs and aspirations of present and future generations.

BOX 2.3

SUSTAINABLE USE (UNEP GLOSSARY, 2007)

Sustainable use

Use in a way and at a rate that does not lead to the long-term degradation of the environment, thereby maintaining its potential to meet the needs and aspirations of present and future generations.

The Protocol contains explicit public participation and access to information provisions. Article 23 specifies that the parties shall promote and facilitate public awareness, education, and participation on issues related to LMOs and biodiversity; that they shall consult with the public in open decision-making processes about LMOs; and that they make the public aware of the information available through the BCH.

The Protocol also contains provisions on **LMO handling, packaging and transportation**. Each contracting party must take the necessary measures to ensure that LMOs subject to intentional transboundary movement within the Protocol's scope are handled, packaged and transported under safety conditions in order to avoid adverse effects on biodiversity conservation and sustainable use (Art 18[1]).

LMO HANDLING, PACKAGING AND TRANSPORTATION
Ensuring that LMOs subject to intentional transboundary movement within the Protocol's scope are handled, packaged and transported under safety conditions in order to avoid adverse effects on biodiversity conservation and sustainable use.

BOX 2.4

TRANSBOUNDARY MOVEMENT (UNEP GLOSSARY, 2007)

Transboundary movement
Movement from an area under the national jurisdiction of one state to or through an area under the national jurisdiction of another state or to or through an area not under the national jurisdiction of any state.

TRANSBOUNDARY MOVEMENT
Movement from an area under the national jurisdiction of one state to or through an area under the national jurisdiction of another state or to or through an area not under the national jurisdiction of any state.

Article 29 of the Protocol includes a governing body, the Conference of the Parties (COP), which serves as the meeting of the parties, to keep under regular review the implementation of the Protocol and make, within its mandate, the decisions necessary to promote its effective implementation.

2.2.3 The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS, 1994)

The SPS Agreement entered into force on 1 January 1995 (with the establishment of the WTO). As of January 2010 the WTO has 153 Members; all Members automatically accede to all multilateral WTO agreements and agree to use the WTO dispute resolution process.

Article 20 of the General Agreement on Tariffs and Trade (GATT) of the WTO allows governments to act on trade in order to protect human, animal or plant life or health, provided they do not discriminate or are used as a disguised protectionism.

AGREEMENT ON THE APPLICATION OF SANITARY AND PHYTOSANITARY MEASURES
Establishes a framework for the protection of food safety, animal and plant health in the context of all sanitary and phytosanitary measures which may directly or indirectly affect international trade.

The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) establishes a framework for the protection of food safety, animal and plant health in this context.

One of the objectives of the SPS Agreement is to encourage the harmonization of **sanitary or phytosanitary measures (SPMs)** on the basis of internationally-accepted scientific standards (Article 3). Because of this, the activity of the recognized standard-setting bodies – Codex, IPPC, and OIE – is central to the SPS Agreement's implementation in the context of food safety, plant and animal life and health, respectively. The Agreement applies to all SPMs which may directly or indirectly affect international trade, and is binding upon all WTO Member States.

SANITARY OR PHYTOSANITARY MEASURES (SPMs)
The SPS agreement provides a multilateral framework of rules to guide the development, adoption and enforcement of sanitary and phytosanitary measures to minimize their negative impacts on trade.

The SPS Agreement also specifically aims to prevent Members from using SPMs as disguised trade restrictions, and notes that they must not create arbitrary or unjustified discrimination among Members where the same conditions exist. However, where conditions differ and, in particular, for developing countries, special provisions apply (Art. 10). "Special and differential treatment" may apply in these cases, allowing longer timeframes for compliance and the potential for exemptions (Art. 10[3]).

The SPS Agreement does not explicitly mention GMOs. However, when GMOs are traded internationally and may pose a threat to human, animal or plant life or health in an importing country, the SPS Agreement applies to national SPMs designed to address the threats prior to import. In general, the Agreement provides a multilateral framework of rules to guide the development, adoption and enforcement of SPMs to minimize their negative impacts on trade (Preamble, Para. 4).

The SPS agreement allows countries to set their own standards, but it also establishes that when these standards are implemented as SPMs they must be applied only to the extent necessary to protect human, animal, plant life or health (Art. 2[1]).

A **Member State's SPMs** must only be applied to the extent necessary, must be based on scientific principles and must not be maintained without sufficient scientific evidence (Art. 2[2]). SPMs must also not arbitrarily or unjustifiably discriminate between Member States where identical or similar conditions prevail and should not be applied in a manner that would constitute a disguised restriction on international trade (Art. 2[3]).

MEMBER STATE SPMs
Must only be applied to the extent necessary, must be based on scientific principles and must not be maintained without sufficient scientific evidence.

The SPS Agreement aims at enhancing trade harmonization among Member States. For this purpose, it establishes that Members should base their SPMs on international standards, guidelines and recommendations (Art. 3[1]). Using accepted international standards allows States to demonstrate that their measures are based on accepted scientific evidence and do not create unnecessary barriers to trade. The Codex Alimentarius, the IPPC and the OIE are recognized in the Preamble as relevant international standard-setting bodies.

Countries wishing to introduce standards and SPMs resulting in a higher level of protection than that offered by an international standard, guideline or recommendation are allowed to do so provided that there is scientific basis to justify the measure (Article 3.3).

Member States must ensure that SPMs are based on assessment of risks to human, animal or plant life or health according to the risk assessment techniques developed by the relevant international organizations (Article 5.1). Measures diverging from the standards adopted by the internationally-recognized organizations, or risk assessments based on techniques different from those elaborated in the framework of these organizations and resulting in greater restrictions on trade must be based on sufficient scientific evidence. Member States can also take relevant **economic factors** into account when assessing risk and establishing risk management measures (Article 5.3).

ECONOMIC FACTORS
Can be taken into account when assessing risk and establishing risk management measures. Economic measures include the potential damage to production or lost sales, the costs of control or eradication of a pest, and the relative cost effectiveness of alternative approaches to limit risks.

Economic measures include the potential damage to production or lost sales, the costs of control or eradication of a pest, and the relative cost effectiveness of alternative approaches to limit risks (Art. 5[3]). Other factors to take into consideration when establishing the appropriate level of protection should include minimizing negative trade effects, avoiding arbitrary or unjustifiable distinctions in the levels a Member State considers appropriate in different situations and ensuring SPMs are not more trade-restrictive than required for an appropriate level of protection (Art. 5 [4-6]).

Member States may provisionally adopt SPMs when scientific evidence for the measures is insufficient (Art. 5[7]). They may seek additional information to enable them to assess any risk in an objective manner and to review the SPM within a reasonable period of time. A Member State can request an explanation from another Member State when the former believes a specific SPM is constraining or could constrain its exports and is not based on an international standard, guideline or recommendation (Art. 5[8]). Members must notify changes in their SPM according to the procedure stipulated in the Annex to the SPS Agreement (Art. 7).

AGREEMENT ON TECHNICAL BARRIERS TO TRADE
Aimed at ensuring that regulations, standards, testing and certification procedures do not create unnecessary obstacles to trade. It is relevant to biotechnology products because it applies to packaging, marking and labelling requirements associated with products resulting from biotechnology.

2.2.4 The Agreement on Technical Barriers to Trade (TBT) (1994)[3]

The Agreement on Technical Barriers to Trade (TBT) is an Agreement signed under the auspices of the WTO. It is aimed at ensuring that regulations, standards, testing and certification procedures do not create unnecessary obstacles to trade. It is relevant to biotechnology products because it applies to packaging, marking and labelling requirements associated with products resulting from biotechnology.

3 Entered into force 1 January 1995 (with the establishment of the World Trade Organization).

The TBT Agreement recognizes countries´ right to adopt the technical regulations and standards they consider appropriate to achieve ¨**legitimate trade objectives**" such as national security, preventing deceptive trade practices, protecting human health or safety, animal or plant life or health, or the environment, consumers' protection and prevention against deceptive practices and other objectives such as quality, technical harmonization or simply trade facilitation, taking account of the risks of non-fulfillment (Art. 2.2). In assessing such risks, relevant elements for consideration are, *inter alia*, available scientific and technical information, related processing technology or intended end-uses of products (Annex, Art. 2.2). They should not cause unnecessary barriers to trade and should be applied equally to national and imported products (Art. 2.1).

LEGITIMATE TRADE OBJECTIVES
Include national security, preventing deceptive trade practices, protecting human health or safety, animal or plant life or health, or the environment, consumers' protection and prevention against deceptive practices and other objectives such as quality, technical harmonization or simply trade facilitation, taking account of the risks of non-fulfillment.

It applies where, for example, a country obliges imported products to include in their labels any traces of GMOs. One of its goals is to encourage the harmonization of technical regulations at international level. To this purpose, it recommends that Members use existing international standards for their national regulations, or for parts of them, unless "their use would be ineffective or inappropriate" to fulfill a given policy objective.

Whenever a technical regulation is based on an international standard, and is applied to achieve one of the legitimate objectives listed, it is presumed not to create an unnecessary barrier to trade (Art. 2.5).

Developing country Member States may adopt technical regulations, standards or conformity assessment procedures aimed at preserving indigenous technology and production methods compatible with their development needs. They are, therefore, not expected to use international standards as the basis to develop technical regulations or standards, which are not appropriate to their development, financial or trade needs (Art. 12.4).

2.2.5 Agreement on Trade-related Aspects of Intellectual Property Rights (TRIPS) (1995)[4]

AGREEMENT ON TRADE-RELATED ASPECTS OF INTELLECTUAL PROPERTY RIGHTS A broad-ranging agreement aimed at ensuring effective and appropriate protection for trade-related intellectual property rights, taking into account differences in national legal systems, and drawing up a multilateral framework of minimum rules to help combat counterfeiting.

The WTO Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) is a broad-ranging agreement aimed at ensuring effective and appropriate protection for trade-related intellectual property rights, taking into account differences in national legal systems, and drawing up a multilateral framework of minimum rules to help combat counterfeiting. TRIPS harmonizes all earlier intellectual property conventions and treaties such as the Paris Convention, the Berne Convention, the Rome Convention, the Treaty on Intellectual Property in Respect of Integrated Circuits and to some extent the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The basic principles include national and most favoured nation treatments, rights of priority and independence of patent. The principle of "independence of patents" is recognized by Article 4bis(1) of the Paris Convention that states that "[p]atents applied for in the various countries... shall be independent of patents obtained for the same invention in other countries...." (Paris Convention).

While TRIPS does not directly relate to biosafety, it interacts with other international agreements on biosafety, notably the ITPGRFA (see section 2.2.8.) and the provisions of the CPB and CBD that address technology transfer, farmers' rights, and access and benefit-sharing (ABS).

PATENT PROTECTION is required to be provided by Member States of the TRIPS Agreement for at least 20 years for inventions, whether products or processes, subject to certain exclusions.

The TRIPS Agreement requires Member States to provide **patent protection** for at least 20 years for inventions, whether products or processes, subject to certain exclusions. It also requires that patents in any field of technology be available without discrimination as to the place of invention and whether products are imported or locally produced (Art. 27.1).

4 Entered into force 1 January 1995 (with the establishment of the World Trade Organization).

There are limited exceptions to the basic rule on patentability. One is to protect human, animal or plant life or health or to avoid serious harm to the environment. Commercial exploitation of an invention in this category must also be prevented and this prevention must be necessary for the protection of *ordre public* or morality, and not simply because exploitation of the invention is prohibited (Art. 27.2).

Another exemption is for plants and animals other than micro-organisms and processes for the production of plants or animals other than non-biological and microbiological processes. However, Member States must still provide either patent protection or an effective *sui generis* system of protection (Art. 27.3[b]). This Article has generated a great deal of debate, and the TRIPS Council continues to discuss how to apply it, and particularly how it relates to the CBD.

2.2.6 The International Plant Protection Convention (IPPC) (1997)[5]

THE INTERNATIONAL PLANT PROTECTION CONVENTION
An international treaty to secure action to prevent the spread and introduction of pests of plants and plant products, and to develop and promote appropriate phytosanitary measures for their control.

The International Plant Protection Convention (IPPC) was originally adopted in 1951. It was subsequently revised in 1997 and came into force in October 2005. It is governed by the Commission on Phytosanitary Measures (CPM), which adopts International Standards for Phytosanitary Measures (ISPMs). The WTO SPS recognizes the IPPC as the organization providing international standards related to plant protection. An SPM that conforms to an international standard established by the IPPC is "deemed to be necessary to protect plant life or health" and "presumed to be consistent" with the SPS Agreement. In this way, government measures to protect plant health are harmonized and are not used as unjustified barriers to trade.

The IPPC is an international treaty to secure action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate

5 Entered into force (latest revision) on 2 October 2005. As of January 2010, 172 contracting parties.

measures for their control. It includes provisions to regulate movements of any organism, object or material capable of harbouring pests or spreading pests that affect plants or plant products (Art. I[4]). The IPPC provides a framework to develop and apply harmonized phytosanitary measures through the elaboration of international standards. It includes an obligation for every member country to designate a national plant protection organization in charge of implementing the Convention at national level and to serve as focal point for other member countries.

PESTS Defined as "any species or biotype of plant, animal or pathogenic agent injurious to plants or plant product". Therefore, the IPPC's scope of application is broad enough to include GMOs, LMOs or products of modern biotechnology that may directly or indirectly damage plants.

"Pests" are defined as *"any species or biotype of plant, animal or pathogenic agent injurious to plants or plant product"* (Art. II[1]). Therefore, the IPPC's scope of application is broad enough to include GMOs or products of modern biotechnology that may directly or indirectly damage plants. Damage to plants is not necessarily limited to cultivated plants. The IPPC can be interpreted to apply to all plants – whether cultivated or wild.

PHYTOSANITARY MEASURES Must meet minimum requirements; they must be non-discriminatory, be necessitated by phytosanitary considerations, proportional and technically justified.

The IPPC provides that phytosanitary measures can be taken for quarantine pests and regulated non-quarantine pests, but not non-regulated pests (Art. VI). **Phytosanitary measures** must meet minimum requirements: they must be non-discriminatory, be necessitated by phytosanitary considerations, proportional and technically justified. They must represent the least trade restrictive measures available and should result in the minimum impediment to the international movement of people, commodities and conveyances (Arts. VI[1] and VII[2][g]). Emergency measures are justified but must be evaluated as soon as possible to justify their continued application (Art. VII[6]). In general, import requirements must comply with minimum stakeholder related requirements between IPPC parties. Some of these include publication and transmission of import requirements, explanation of the rationale for restrictions, promptness of review, and revision of provisions when appropriate (Art. VII[2]).

The Commission on Phytosanitary Measures (CPM) of the IPPC (and previously the Interim Commission on Phytosanitary Measures) has developed a number

of **International Standards for Phytosanitary Measures (ISPM)**. Of special relevance for biotechnology is ISPM No. 11, "Pest risk analysis for quarantine pests, including analysis of environmental risks and LMOs". Annex 2 of ISPM 11 states that phytosanitary risks that may be associated with LMOs are within the scope of the IPPC, and should be considered using pest risk analysis (PRA), as described in the body of the ISPM. Annex 3 gives guidance on determining what factors associated with characteristics or properties related to the genetic modification might create the potential for phytosanitary risks from an LMO.

INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES (ISPM)
Concerning biotechnology: ISPM 11, stating that phytosanitary risks that may be associated with LMOs are within the scope of the IPPC, and should be considered using pest risk analysis (PRA).

A supplement to ISPM 11 Annex 3 published in 2003 adds definitions and gives further guidance on conducting risk assessments for LMOs, noting that those LMOs will not have the characteristics of a potential pest and will therefore not warrant a complete PRA. It suggests three potential pathways for an LMO to present a pest risk: (1) the organism itself; (2) the combination of genetic material; and (3) the consequences of moving genetic material (Annex III[1]). Section 1.15 provides additional details on assessing the potential of an LMO to become a pest. Additional guidance on assessing economic risks is provided in section 2.3.

2.2.7 The Convention on Access to Information, Public Participation in Decision-Making and Access to Justice in Environmental Matters (Aarhus Convention) (1998)[6]

THE AARHUS CONVENTION
An environmental agreement intended to link environmental and human rights, with a focus on the needs of future generations and a belief that sustainable development requires broad stakeholder involvement.

The Convention on Access to Information, Public Participation in Decision-Making and Access to Justice in Environmental Matters is a regional convention developed by Members of the United Nations Economic Commission for Europe (UNECE) and Members with consultative status with the Economic Commission for Europe (ECE). It is more commonly known as the Aarhus Convention.

6 Entered into force (latest revision) on 2 October 2005. As of January 2010, 172 contracting parties.

The Aarhus Convention is an environmental agreement intended to link environmental and human rights, with a focus on the needs of future generations and a belief that sustainable development requires broad stakeholder involvement. It highlights that government transparency and accountability are necessary for environmental protection. To that end, it addresses requirements for governments to create processes and methods for public participation in the negotiation and implementation of international environmental agreements.

The UNECE puts it thus: "The subject of the Aarhus Convention goes to the heart of the relationship between people and governments. The Convention is not only an environmental agreement, it is also a Convention about government accountability, transparency and responsiveness."

The Aarhus Convention grants the public rights and imposes on parties and public authorities obligations regarding access to information and public participation and access to justice (http://www.unece.org/env/pp/).

ACCESS TO ALL INFORMATION must be made available to the public by the competent national authority regarding decision-making processes. The public must be allowed to submit any comments, information, analyses or opinions considered relevant to the proposed activity.

The parties to the Aarhus Convention established a working group on GMOs in 2002 (Decision I/4). This working group prepared the "Guidelines on Access to Information, Public Participation and Access to Justice with respect to Genetically Modified Organisms" adopted in 2003.

The Convention is premised upon the principle that every person of present and future generations has the right to live in an environment adequate to his or her health and wellbeing. To that end, governments should guarantee the rights of access to information, public participation in decision-making and access to justice in environmental matters (Art. 1).

Competent national authorities must give the public **access to all information** relevant to the decision-making, subject to certain exceptions. The public must

be allowed to submit any comments, information, analyses or opinions considered relevant to the proposed activity.

The Convention addresses GMOs in the context of decision-making in Article 6(11). Following the modification introduced by Decision II/1 in 2005, Article 6 introduces a new system of "early and effective information and public participation prior to making decisions on whether to permit the deliberate release into the environment and placing on the market of genetically modified organisms." Per a legal opinion from the UN Office of Legal Affairs (available at http://www.unece.org/env/pp/gmo/Memo_LDJ_draft_9_Jan08.tif), it is likely that the Addendum only applies to Members who have signed it (25 as of January 2010).

The Convention establishes mechanisms for **public participation in decisions** on the deliberate release into the environment and placing on the market of GMOs with an adequate time frame, and requires that these provisions be mutually supportive of national biosafety frameworks and CPB requirements (Article 6).

PUBLIC PARTICIPATION
In decisions on the deliberate release into the environment and placing on the market of genetically modified organisms with an adequate time frame must be ensured.

Exceptions to these requirements are admitted for products already approved or for research use or culture collections approved through national biosafety regulatory frameworks and for which adequate experience exists in comparable ecosystems (Annex 1.bis).

The Aarhus Convention also specifically references the CPB and calls on its Members to ratify or accede to the CPB, but notes that the Aarhus Convention still provides an appropriate framework for public participation regarding GMOs.

2.2.8 The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (2004)[7]

INTERNATIONAL TREATY ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE
The main objectives of the Treaty are the conservation and sustainable use of plant genetic resources for food and agriculture (PGRFA) and the fair and equitable sharing of the benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security.

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) applies to all plant genetic resources relevant for food and agriculture. The main objectives of the Treaty are the conservation and sustainable use of plant genetic resources for food and agriculture (PGRFA) and the fair and equitable sharing of the benefits arising out of their use, in harmony with the CBD, for sustainable agriculture and food security (Art. 1).

"**Plant genetic resources for food and agriculture**" are defined as "any genetic material of plant origin of actual or potential value for food and agriculture" (Art. 2). The Treaty's application to GMOs is not direct. The term "modern biotechnologies" is only referred to once in the preamble: "plant genetic resources for food and agriculture are the raw material indispensable for crop genetic improvement, whether by means of farmers' selection, classical plant breeding or modern biotechnologies, and are essential in adapting to unpredictable environmental changes and future human needs."

PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE
Are defined as "any genetic material of plant origin of actual or potential value for food and agriculture."

State parties are obliged to assess, minimize or eliminate any threats to PGRFA and to promote both *in situ* conservation and the compilation of genetic resources for preservation in public collections (Art. 5). State parties should further promote or support, as appropriate, farmers' and local communities' efforts to manage and conserve on-farm their PGRFA. This could include the use of modern biotechnologies. The Treaty mandates that parties develop and maintain measures to advance the sustainable use of plant genetic resources such as extending the genetic base of crops available to farmers and supporting plant breeding efforts that strengthen the capacity to develop varieties adapted to particular ecological conditions.

7 Entered into force 29 June 2004. As of January 2010, 120 parties.

The contracting parties recognize the enormous contribution that the **local and indigenous communities** and farmers of all regions of the world, particularly those in the centres of origin and crop diversity, have made and will continue to make for the conservation and development of plant genetic resources which constitute the basis of food and agriculture production throughout the world. To that end, the Treaty confers responsibility on governments to implement farmers' rights which include the protection of traditional knowledge relevant to PGRFA, the right to equitably participate in sharing benefits arising from their utilization and national decision-making about genetic resources (Art. 9). Farmers have the right to save, use, exchange and sell farm-saved seed/propagating material, but this is made subordinate to national law (Art. 9.3).

LOCAL AND INDIGENOUS COMMUNITIES particularly those in the centres of origin and crop diversity, have made and continue to make enormous contributions to the conservation and development of plant genetic resources which constitute the basis of food and agriculture production throughout the world.

State parties commit to the establishment of an efficient, effective and transparent multilateral system for access to and benefit sharing of PGRFA in a fair and equitable way and on a complementary and mutually reinforcing basis (Art. 10). The multilateral system applies to over sixty-four major crops and forages important for food security listed in Annex I to the Treaty that are under the control of the contracting parties and in the public domain (Art. 11). The contracting parties agree that **benefits arising from the use of PGRFA** that are shared under the multilateral system should flow primarily, directly and indirectly, to farmers in all countries, especially in developing countries and countries with economies in transition, who conserve and sustainably utilize PGRFA.

BENEFITS ARISING FROM THE USE OF PGRFA should flow primarily, directly and indirectly, to farmers in all countries, especially in developing countries and countries with economies in transition, who conserve and sustainably utilize PGRFA.

Article 12 stipulates conditions to the access to plant genetic resources for food and agriculture under the multilateral system. Resources may be obtained solely for the purpose of utilization and conservation for research, breeding and training for food and agriculture, provided that such purpose does not include chemical, pharmaceutical or other non-food/feed industrial uses. The Treaty makes provision for the payment of an equitable share of the monetary benefits where a commercial product is developed using plant genetic resources accessed under the multilateral

system and the product is not available without restriction for further research or breeding. Payment is voluntary if others may use it for further research and breeding. A standard material transfer agreement prepared by the Governing Body sets the terms and conditions for ABS.

2.3 NON-BINDING INSTRUMENTS ON BIOSAFETY

NON-BINDING INSTRUMENTS ON BIOSAFETY
Non-binding agreements have often created the context and formed the basis for later binding agreements on biosafety: on other occasions, they have been "*de facto*" implemented by countries.

As is the case for binding instruments, non-binding international instruments may also address biosafety directly or address provisions related to GMOs within a broader scope. Non-binding agreements have often created the context and formed the basis for later binding agreements on biosafety; on other occasions, they have been "de facto" implemented by countries. This is the case for certain Organisation for Economic Co-operation and Development (OECD) and United Nations Environment Programme (UNEP) recommendations specifically addressing biosafety considerations, such as Agenda 21, Chapter 16. A number of other instruments seek to prevent the establishment of invasive species through guidelines on transportation, import, and release of living organisms. These include the United Nations Industrial Development Organization (UNIDO) Code of Conduct for the Release of Organisms into the Environment, and, with respect to the potential release into the environment of transgenic aquaculture species, the FAO Code of Conduct for Responsible Fisheries.

2.3.1 The Organisation for Economic Co-operation and Development (OECD) Safety Considerations for Biotechnology (1992)

THE OECD SAFETY CONSIDERATIONS FOR BIOTECHNOLOGY
Two issues are addressed: best practices for biotechnological industrial production for fermentation-derived products of biotechnology and Good Developmental Principles (GDPs) for field research with plants and microorganisms with newly introduced traits.

The 1992 Organisation for Economic Co-operation and Development (OECD) Safety Considerations follow earlier OECD work in 1986 that set out the first safety guidelines for biotechnology applications to industry, agriculture and the environment. The 1986 Recombinant-DNA Safety Considerations provided guidance to

be used in assessing field research involving GMOs. The 1992 Safety Considerations address two issues: best practices for biotechnological industrial production for fermentation-derived products of biotechnology and **Good Developmental Principles (GDPs)** for field research with plants and micro-organisms with newly introduced traits.

The Safety Considerations are intended to ensure the environmental safety of small-scale basic and initial applied research involving genetically modified plants and micro-organisms. The GDPs provide guidance to researchers on selecting organisms, choosing the research site and designing appropriate experimental conditions. They recommend step-by-step evaluation of new products, where knowledge is limited, and small-scale experiments before conducting large-scale or commercial growing operations. The Safety Considerations highlight three key factors: (1) characteristics of the organism; (2) characteristics of the research site; and (3) experimental conditions. Annex 1 provides particular scientific considerations for small-scale research with plants, including unintentional spread of plants (with the analogy of invasive species) and plant-produced toxins.

GOOD DEVELOPMENTAL PRINCIPLES (GDPs)
Provide guidance to researchers on selecting organisms, choosing the research site and designing appropriate experimental conditions. They recommend step-by-step evaluation of new products where knowledge is limited, and small-scale experiments before conducting large-scale or commercial growing operations.

BOX 2.5

AGENDA 21 (UNEP GLOSSARY, 2007)

Programme of action on sustainable development adopted [by more than 178 governments] at the UN Conference on Environment and Development [held in Rio de Janeiro, Brazil] in 1992, often referred to as the "Blueprint for Sustainable Development." Agenda 21 has 40 chapters dealing with all aspects of sustainable development, including social and economic dimensions (combating poverty and promoting human health), conservation and resource management, major groups (e.g. women, indigenous people, business and unions), and means of implementation (e.g. financial resources, transfer of technology, public awareness and education).

2.3.2 Agenda 21, Chapter 16 (1992)

AGENDA 21
Sets out a five-point programme: "(a) increasing the availability of food, feed and renewable raw materials; (b) improving human health; (c) enhancing environmental protection; (d) enhancing safety and developing international mechanisms for co-operation; and (e) establishing enabling mechanisms to develop and apply biotechnology in an environmentally sound manner".

Agenda 21 addresses the environmentally sound management of biotechnology in Chapter 16. The programme is to help foster the application of internationally agreed environmentally sound management of biotechnology principles to ensure environmentally sound management; to engender public trust and confidence; to promote development of sustainable biotechnological applications; and establish appropriate enabling mechanisms (Chapter 16.1).

Agenda 21 sets out a five point programme: "(a) increasing the availability of food, feed and renewable raw materials; (b) improving human health; (c) enhancing environmental protection; (d) enhancing safety and developing international mechanisms for co-operation; and (e) establishing enabling mechanisms to develop and apply biotechnology in an environmentally sound manner" (16.1). This programme encourages the development of biotechnology that can assist developing countries as well as industrialized countries, noting that early benefits from biotechnology accrued mainly to the latter. It suggests research into applications that increase food and feed supply and reduce environmental degradation.

At the same time, it notes that food supply questions are also related to food distribution problems, and highlights the importance of taking into account the needs of farmers; the socio-economic, cultural and environmental impacts; the need to promote sustainable social and economic development while paying particular attention to how the use of biotechnology will affect the maintenance of environmental integrity (Chapter 16.4).

The basis for action on programme area "d" includes the need for internationally agreed principles on risk assessment and management; adequate and transparent safety and border-control procedures; the primary consideration of the organism in safety assessment; the application of the principle of familiarity

in a flexible framework considering national requirements, and a step-by-step and case-by-case approach; the evolution to a more comprehensive approach based on the experiences; complementary consideration of risk assessment and risk management; and classification into contained use and release into the environment (Chapter 16.29).

BOX 2.6

PRINCIPLE OF FAMILIARITY (NAP *ET AL.*, 2003)

"[Familiarity] can be considered the ecological counterpart of the concept of 'substantial equivalence', although in some publications these two concepts are also considered separately for environmental release. Familiarity considers whether the GM plant is comparable to its traditionally bred counterpart in environmental safety. Such comparison may assess the relevant issues in a GM crop without direct experience. Familiarity considers the biology of the plant species, the trait introduced, and the agricultural practices and environment used for crop production, in comparison with a suitable counterpart, often the parental non-GM crop; the aim is to establish if the GM change presents any new or greater risks relative to the counterpart. This allows a relative level of safety to be established for the GM crop."

PRINCIPLE OF FAMILIARITY
Familiarity considers whether the GM plant is comparable to its traditionally bred counterpart in environmental safety.

The aim of the programme area is "to ensure safety of biotechnology development, application, exchange and transfer through international agreement on principles to be applied on risk assessment and management, with particular reference to health and environment considerations, including the widest possible public participation and taking into account ethical considerations" (Chapter 16.30). To manage biotechnology, governments should make existing safety procedures widely available and adapt them to local needs; further develop existing safety

procedures; compile a framework of internationally agreed principles as a basis for guidelines on biosafety; and exchange information on safety procedures and assist in emergency situations (Chapter 16.32).

STRENGTHENED ENDOGENOUS CAPACITIES IN DEVELOPING COUNTRIES Are required in order to facilitate accelerated development and application of biotechnology. This includes the need for socio-economic assessment and safety assessment, as well as national mechanisms to allow for informed comment by the public with regard to biotechnology research and application.

Programme area "e" stresses the need for **strengthened endogenous capacities in developing countries** in order to facilitate accelerated development and application of biotechnology. This includes the need for socio-economic assessment and safety assessment, as well as national mechanisms to allow for informed comment by the public with regard to biotechnology research and application. The basis for action also recognizes that biotechnological research and its application could have significant positive and negative socio-economic and cultural impacts and that these should be identified early in the development phase to appropriately manage them. One of the programme area objectives is to raise public awareness on risks and benefits related to biotechnology (16.37-39).

2.3.3 The United Nations Environment Programme (UNEP) Technical Guidelines on Biosafety (1995)

THE UNEP TECHNICAL GUIDELINES ON BIOSAFETY Provide the possibility for states to voluntarily develop mechanisms for evaluating the biosafety of "organisms with novel traits," those whose genetic make-up is unlikely to develop naturally, and to identify, assess and manage the risks associated with the use of biotechnology.

The UNEP Guidelines were adopted in 1995. They were designed and adopted as a contribution to the implementation of Agenda 21, Chapter 16. They provide the possibility for states to voluntarily develop mechanisms for evaluating the biosafety of "organisms with novel traits," those whose genetic make-up is unlikely to develop naturally, and to identify, assess and manage the risks associated with the use of biotechnology. The Guidelines acknowledge the importance of assessing socio-economic and other impacts of new biotechnologies but do not address these issues.

The Guidelines focus on human health and environmental safety for all applications of biotechnology, whether research, development or commercialization. Section II (18-27) addresses general considerations for managing applications of

biotechnology, while Section III (28-32) deals with risk assessment and risk management. The Guidelines suggest a process of hazard identification, risk assessment, and risk management.

Risk assessment and risk management can be based in part on knowledge and experience with an organism (familiarity) with the proviso that familiarity does not imply that an organism is safe, while unfamiliarity does not imply that an organism is necessarily unsafe. **Unfamiliarity** means, however, that organisms should be assessed on a case-by-case basis. With experience and knowledge, a risk assessment may apply to a group of organisms for characteristics functionally equivalent on a physiological level, and monitoring is important to gain this knowledge and experience.

UNFAMILIARITY
Unfamiliarity does not imply that an organism is necessarily unsafe; however, that organisms should be assessed on a case-by-case basis.

The development of generic risk assessment approaches or exemptions in one country does not necessarily mean that other countries will apply similar approaches. The user of the organism has the primary responsibility for the safe use or transfer of organisms with novel traits once adequate risk management strategies have been devised. The introduction of organisms with novel traits into centres of origin must be particularly considered in risk assessment and management.

The Guidelines reflect the principle that risk management should be proportional to the level of risk and the scale of the operation. Risk management measures should be taken until risks have been minimized to acceptable levels. If risk cannot be minimized either the intended operation should not proceed, or a risk/benefit analysis could be used to determine whether the higher level of risk is acceptable.

Risk assessment and management need to be undertaken by the competent authorities at national or regional level. The oversight authorities are responsible for encouraging public participation and access to information on which decisions are based.

Confidential information should be respected. The Guidelines require notification to be made to a potentially affected country where any transboundary impacts occur or where any adverse effects could affect it (Section IV, Paras. 33-39).

2.3.4 The United Nations Industrial Development Organization (UNIDO) Code of Conduct for the Release of Organisms into the Environment (1991)

THE UNIDO CODE OF CONDUCT FOR THE RELEASE OF ORGANISMS INTO THE ENVIRONMENT
Provides general principles governing standards of practice for all parties involved with the introduction of organisms or their products/metabolites into the environment.

The UNIDO Code of Conduct for the Release of Organisms into the Environment provides general principles governing standards of practice for all parties involved with the introduction of organisms or their products/metabolites into the environment (Sec. II[A][1][a]). It covers GMOs in all stages of research, development and disposal while focusing on release into the environment (Sec. I[B]).

The Code is founded upon a number of general principles. For example, Section II(C) addresses regulatory oversight and risk assessment, distinguishing process from product. The Code suggests that risk assessment should be focused on the characteristics of the resulting product rather than the molecular or cellular techniques used to produce it. Furthermore, safety precautions and monitoring procedures should be proportional to the level of assessed risk.

National authorities, industries and researchers have the responsibility to make safety information available to the public. Any unexpected or adverse public health or environmental impacts related to the GMO should be reported to appropriate authorities at national and international levels. Risk assessment should be based on "sound scientific principles" involving the participation of experts from appropriate disciplines. Systems to review proposed applications should remain flexible and adaptable in relation to the latest scientific information. Information on anticipated consequences, which may be transboundary in nature, needs to be provided to those countries that may be affected.

The **actions and responsibilities of governments** include assuring the independence of the assessment process, the use of multi-disciplinary scientific competence and using case-by-case evaluation as the rule unless sufficient experience and an adequate body of knowledge is gathered to allow classifications and general experience on GMO behaviour. Researchers have the general responsibility of evaluating risks at appropriate research and development stages. Approvals should be secured prior to the conduct of any activity involving release and unexpected or adverse impacts on public health or the environment should be notified to the appropriate national authorities. The applicant should notify and suggest alternative review mechanisms to national authorities where a regulatory procedure is not yet in place.

ACTIONS AND RESPONSIBILITIES OF GOVERNMENTS
Include assuring the independence of the assessment process, the use of multi-disciplinary scientific competence and using case-by-case evaluation as the rule unless sufficient experience and an adequate body of knowledge is gathered to allow classifications and general experience on GMO behaviour.

2.3.5 The FAO Code of Conduct for Responsible Fisheries (1995)

The FAO Code of Conduct for Responsible Fisheries is a voluntary set of principles and standards designed to ensure the effective conservation, management and development of all fisheries with due respect for ecosystems and biodiversity. It is global in scope and applies to all governments, fisheries organizations, non-governmental organizations and the private sector (Preface, Art. 1).

THE FAO CODE OF CONDUCT FOR RESPONSIBLE FISHERIES
Is a voluntary set of principles and standards designed to ensure the effective conservation, management and development of all fisheries with due respect for ecosystems and biodiversity.

In its list of general principles (Art. 6), the Code states that conservation and management decisions should be based on the best scientific evidence, taking into account traditional knowledge, as well as environmental, economic and social factors. Furthermore, the precautionary approach is to be applied to the conservation, management and development of living aquatic resources.

The Code's **aquaculture provisions** (addressed in Article 9) address the release of GMOs in the context of aquaculture operations. In accordance with the principle of "responsible development of aquaculture" (Article 9.2) government authorities, aquafarmers and fishery managers have a special obligation to minimize the risks

AQUACULTURE PROVISIONS
Address the release of GMOs in the context of aquaculture operations.

of introducing non-native species or genetically altered stocks used for aquaculture or culture-based fisheries into waters where there is a significant risk of their spreading into the waters of other states.

The use of aquatic genetic resources for the purposes of aquaculture, including culture-based fisheries, is further addressed in Article 9.3, which introduces the duty of the states to conserve genetic diversity and maintain integrity of aquatic communities and ecosystems by appropriate management. States should also conserve genetic diversity and maintain the integrity of aquatic communities and ecosystems. Specifically, states are to minimize the harmful effects of introducing "genetically altered stocks" used in aquaculture, including culture-based fisheries, into waters. This is especially important where there is significant potential for these stocks to spread into the waters of other states.

2.3.6 The Codex Alimentarius (Codex)[8]

THE CODEX ALIMENTARIUS
Is a collection of internationally adopted food standards presented in a uniform manner. The Codex Commission has been recognized as an international standard setting body for purposes of implementing the World Trade Organization's Agreement on Sanitary and Phytosanitary Measures.

The Codex Alimentarius (Codex) is a collection of internationally adopted food standards presented in a uniform manner. The Codex Commission has been recognized as an international standard setting body for purposes of implementing the WTO's Agreement on Sanitary and Phytosanitary Measures (SPS Agreement).

The purpose of the Codex Alimentarius Commission is to protect the health of consumers, to ensure fair practices in food trade, and to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations. The Commission's Medium-term Objectives include *inter alia* "consideration of standards, guidelines or other recommendations as appropriate for foods derived from biotechnology or traits introduced into foods

8 Codex instruments are available for review at the Codex website for current official standards (http://www.codexalimentarius.net/web/standard_list.jsp.)

by biotechnology on the basis of scientific evidence and risk-analysis and having regard, where appropriate, to other legitimate factors relevant for the health protection of consumers and promotion of fair practices in food trade."

The Codex Alimentarius Commission includes an *Ad Hoc* Intergovernmental Task Force on Foods derived from Biotechnology that was created in 1999. In 2003, the Codex Commission adopted three **standards on foods derived from biotechnology**: "Principles for the risk analysis of foods derived from modern biotechnology;" "Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants;" and "Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms."

STANDARDS ON FOODS DERIVED FROM BIOTECHNOLOGY
Adopted by the Codex Commission; includes "Principles for the risk analysis of foods derived from modern biotechnology;" "Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants;" and "Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms."

These standards establish overarching principles for the risk analysis of foods derived from modern biotechnology and the food safety assessment of foods derived from recombinant DNA plants and micro-organisms. The principles dictate a premarket assessment, performed on a case-by-case basis and including an evaluation of both direct effects (from the inserted gene) and unintended effects (that may arise as a consequence of insertion of the new gene).

It should be noted that Codex standards apply to all types of foods and, for this reason, the Codex will need to deal with foods of plant, animal, and fish origin. The impact of feeding GMO plants to animals, and the nature of the resulting foods from these animals will also need to be addressed.

As part of its work, the Codex Commission also keeps under review its relationship with other international intergovernmental organizations such as the Convention on Biological Diversity (CBD) and the Cartagena Protocol on Biosafety (CPB).

2.3.7 Office International des Epizooties (OIE) (World Organization for Animal Health) (1924)

OFFICE INTERNATIONAL DES EPIZOOTIES (OIE) is the world organization and standard setting body responsible for animal health.

The OIE, established in 1924, is the world organization and standard setting body responsible for animal health. It has three main objectives: (1) to inform governments of the occurrence and course of animal disease and of ways to control disease outbreaks; (2) to coordinate international scientific research on the surveillance and control of animal disease and (3) to facilitate the harmonization of regulations pertaining to trade in animals and animal products.

Among its activities, the OIE establishes standards that member countries should adopt to protect themselves from diseases, without setting up unjustified sanitary barriers, and to ensure the safety of animals and animal products in transboundary movements and trade. The main normative instruments produced by the OIE are the International Animal Health Code for terrestrial animals, the Manual of Standards for Diagnostic Test and Vaccines, the International Aquatic Animal Health Code and the Diagnostic Manual for Aquatic Animal Diseases.

The OIE cooperates with the Codex Alimentarius Commission and plays an important role in acting "upstream" from other food safety quality standard setting organizations in suggesting norms, guidelines, and recommendations.[9]

In 2005, the International Committee adopted Resolution (XXVIII) on "Applications of Genetic Engineering for Livestock and Biotechnology Products". An *Ad Hoc* Group on Biotechnology was created and, in August 2008, was divided into two new ad hoc groups, one to focus on molecular diagnostics and the other on vaccines related to new and emerging biotechnologies (the *Ad Hoc* Group on Molecular Diagnostics and the *Ad Hoc* Group on Vaccinology, respectively (OIE, 2008)).

9 Resolution No. XXV, recommending that the APFSWG's 2008/2009 work programme guide the OIE's animal production food safety activities

2.4 OTHER AGREEMENTS

A number of agreements with subject matters different from biotechnology address some issues related to biotechnology, such as rules on labelling, certification, threshold levels, monitoring and traceability. These provisions may affect how biosafety agreements are interpreted or give guidance in creating legislation specific to biotechnology. Agreements dealing with animal feed may also have bearing on biosafety frameworks.

Additionally, agreements on avoiding damage from invasive species may also have some bearing on biosafety legislation, as they present means of avoiding negative impacts from introduced species. Examples are the RAMSAR Convention on Wetlands, the ASEAN Agreement and African Convention on the Conservation of Nature and Natural Resources, the FAO Code of Conduct for the Import and Release of Exotic Biological Control Agents, the ICES Code of Practice on the Introductions and Transfers of Marine Organisms and the IUCN Guide to Designing Legal and Institutional Frameworks on Alien Invasive Species.

2.5 POTENTIAL OVERLAPS AND CONFLICTS BETWEEN TREATIES

Specific international agreements may create situations that require additional interpretation and careful implementation in relation to other agreements. Several international instruments are complementary or overlap, and members are trying to establish means of working in harmony rather than duplicating efforts. Harmonization of standards is a driving factor in creating international agreements, and institutions continue to seek improved harmonization in the area of biosafety.

Areas of overlap among international instruments include requirements for risk analysis, monitoring and notification. Areas of conflict that may arise in trade situations include questions of import restrictions, labelling, liability, and ABS. This section reviews interactions among specific agreements relating to biosafety.

OTHER AGREEMENTS
A number of agreements with subject matters different from biotechnology address some issues related to biotechnology, such as rules on labelling, certification, threshold levels, monitoring, and traceability. These provisions may affect how biosafety agreements are interpreted or give guidance on creating legislation specific to biotechnology.

POTENTIAL OVERLAPS AND CONFLICTS BETWEEN TREATIES
Specific international agreements may create situations that require additional interpretation and careful implementation in relation to other agreements. Several international instruments are complementary or overlap, and members are trying to establish means of working in harmony rather than duplicating efforts.

BOX 2.7

VIENNA CONVENTION ON THE LAW OF TREATIES (1969)**: PRINCIPLES GOVERNING INTERPRETATION OF SUCCESSIVE TREATIES**

PRINCIPLES GOVERNING INTERPRETATION OF SUCCESSIVE TREATIES
These principles guide the application of successive treaties relating to the same subject matter.

Article 30: Application of successive treaties relating to the same subject matter

2. When a treaty specifies that it is subject to, or that it is not to be considered as incompatible with, an earlier or later treaty, the provisions of that other treaty prevail.

3. When all the parties to the earlier treaty are parties also to the later treaty but the earlier treaty is not terminated or suspended in operation under article 59, the earlier treaty applies only to the extent that its provisions are compatible with those of the later treaty.

2.5.1 Potential areas of conflict: trade concerns

TRADE CONCERNS
Include concerns over what are acceptable considerations when directly limiting or restricting trade in GMOs or LMOs, as well as what measures may indirectly limit or restrict trade in ways that fail to comply with international agreements.

The main area of potential conflict in international biosafety issues concerns trade issues broadly. These relate to concern over what are acceptable considerations when directly limiting or restricting trade in GMOs or LMOs, as well as what measures may indirectly limit or restrict trade in ways that fail to comply with international agreements. There are two different potential areas of disagreement. First, there may be conflicts between what constitutes "science-based" decision-making, what are the proper tools to use to assess risks, and what role precautionary policies can play in decisions. Second, there is the question of how institutions can respond to citizen, producer, and consumer concerns that go beyond direct harm to environmental or human health. Questions of labelling and liability legislation fall into this second category.

In fact, international conflicts have already arisen over restrictions on GMO approvals, as with the United States leading a group that challenged the European Commission (EC) de facto moratorium on GMO approvals at the WTO (see Box 2.8 for a discussion of this case). Other debates exist over efforts to harmonize standards for labelling and the related issues of certification, traceability, and monitoring. Liability and redress standards have been an area of contention in CPB COP/MOP meetings. In addition, there are potential conflicts over ABS and intellectual property rights protection.

Where states are party to two or more potentially conflicting agreements, minimizing conflicts requires careful navigation and interpreting agreements in the most mutually supportive fashion possible (Oberthür and Gehring, 2006).

BOX 2.8

THE WTO BIOTECH DISPUTE (EXCERPTED FROM SPREIJ, 2007)

One of the most awaited cases in WTO history has undoubtedly been the *Biotech* dispute. Because of its complexity, the dispute encountered several delays but on 29 September 2006,(...) the Panel Report was issued to the public. It was the lengthiest report in WTO history. Publication of the report was followed by much debate, in particular within the EC, which eventually decided not to appeal the report. On 21 November 2006, as mentioned above, the DSB formally adopted the report.[...]

In the beginning of the 1990s, in accordance with its legislation, the EC authorized a number of GMOs for commercial release into the environment for different uses, some for cultivation, others as food or feed. By the mid-90s, however, several EC Member States started to express concerns. They believed that the existing

THE WTO BIOTECH DISPUTE
Relates to a dispute over trade restrictions between the EC, which had established a de facto moratorium on GMO approvals, and major GMO producers such as the USA, Canada and Argentina.

regulatory framework was not adequate, in particular with regard to issues such as risk assessment, labelling and traceability. As a result of these concerns, and in reaction to rapid scientific developments and the negotiation of the Protocol, no new GMOs were approved under the legislation in force during the period October 1998 until May 2004. By that time, the EC had adopted a new set of rules (...)

However, in August 2003, just a few weeks before the Protocol entered into force, the United States, Canada and Argentina, all major GMO producers and exporters, requested the establishment of a panel under the WTO dispute settlement procedure. In short, the countries claimed that:

- the EC had implemented a general de facto moratorium;
- the EC had failed to approve specific GM products;
- the EC Member States had prohibited products which had been approved by the EC after consideration by its own scientific regulatory approval process;
- the moratoria and the national prohibitions constituted an unjustified barrier to their trade in agricultural and food products, thus violating the SPS Agreement as well as GATT. Some of the complaints also alleged violations of the TBT Agreement.

The panel analysed the scope of the SPS Agreement and found that the EC approval procedures were - in fact - SPS measures.

It also found that the EC had "de facto" established a moratorium, however that this moratorium was not an SPS measure *per se* but rather affected the operation and application of the EC approval procedures. In addition, it found that the EC's failure to complete its approval procedures without "undue delay" was inconsistent with the Agreement's provisions on control, inspection and approval procedures (Article 8 and Annex C).

The panel also ruled on the prohibitions that a number of EC Member States – Austria, France, Germany, Greece, Italy, Luxembourg and the United Kingdom- had imposed on the importation, marketing or sale of a number of biotech products which had already been approved at Community level. The panel found that these prohibitions were also SPS measures and could not be regarded as provisional SPS measures (Article 5.7) - as the EC had argued - because there was sufficient scientific evidence available to conduct a risk assessment. In fact, risk assessments had been conducted under the EC scientific regulatory approval process and resulted in positive opinions. Consequently, the prohibitions were not based on these risk assessments and although some Member States submitted additional reports and studies, the panel considered that the additional documentation did not constitute a proper risk assessment. These prohibitions thus violated the SPS Agreement (Article 5.1).

Of particular interest is that the panel took a wide view of the SPS Agreement and found that a broad range of measures to protect biodiversity fall within its scope, including cross-contamination of plants by GM plants, reduction of the economic value of crops, effects on non-target insects and plants, etc. The panel considerations on the applicability of the SPS Agreement are contained in paragraphs 7.147 to 7.437 of the report.

The panel also addressed the issue of the application of the CBD and the Protocol (paragraphs 7.49 to 7.96). Generally, claims under the WTO dispute settlement mechanism can only be based upon violation of WTO Agreements but - under certain circumstances - other international agreements can be taken into account in the interpretation of WTO Agreements or be used as a defence. For instance, a country can admit to have violated the SPS Agreement but declare that it did so because it had to implement another international

agreement to which it is a party. The panel considered that if a rule of international law is not applicable to one of the parties to the dispute, it is not applicable in the relations between all WTO Members.

Given that the United States was not a party to the CBD, the panel ruled that it was not required to take the CBD into account in interpreting the WTO Agreements at issue in the dispute. Similarly, the panel considered that it was not required to take the Protocol into account since Argentina, Canada and the United States were not parties to it. Moreover, the panel noted that the Protocol had entered into force after the panel was established.

Apart from the panel findings on the applicability of the SPS Agreement, it should be noted that the report in itself is a narrow and specific ruling. The panel did not rule on a number of important questions that remain outstanding.

For instance, it did not examine:

- whether biotech products in general are safe or not;
- whether the biotech products at issue in the dispute are "like" their conventional counterparts; Although this claim was made by the complaining parties in relation to some aspects of their complaints, the panel did not find it necessary to address those aspects of the complaints since the EC and the Member States violated the SPS Agreement; the thorny "like" issue would certainly have come up in considering violations of the TBT Agreement and/or GATT.
- whether the EC has a right to require pre-marketing approval of biotech products;
- whether the EC approval procedures are consistent with the EC's obligations under the WTO Agreements;
- the conclusions of the relevant EC scientific committees regarding the safety evaluation of specific biotech products.

2.5.2 Interactions among specific agreements

The multilateral WTO agreements that form part of international biosafety frameworks – GATT, TBT, and in particular TRIPS and SPS – are the ones that have the most complex interrelationships with other instruments. The WTO is an unusual international instrument in that it has its own dispute resolution mechanism, which sets up a dispute resolution panel to deliver a binding verdict on disputes between or among Members.

INTERACTIONS AMONG SPECIFIC AGREEMENTS
The multilateral WTO agreements that form part of international biosafety frameworks – GATT, TBT, and in particular TRIPS and SPS – are the ones that have the most complex interrelationships with other instruments.

The WTO relies on certain other international instruments to serve as standard-setting instruments. If regulations are in compliance with these other instruments, they are assumed to be in compliance with WTO rules, as well. Codex Alimentarius and the IPPC have this standard-setting relationship with the WTO for food safety and plant health standards, respectively, while the OIE addresses animal health and trade in animals and animal products. OIE also informs the Codex.

Interpretations of the SPS could generate **conflict with interpretations** of other international instruments, particularly the CBD and CPB, as well as national biosafety frameworks. As the SPS is predicated on "science-based" risk assessment and strictly limits precautionary decision-making, it may come into conflict with biosafety instruments based on precautionary approaches. SPS Article 5(7) states that inadequacy of available data for decisions may allow states to adopt provisional SPM, but only if they actively seek the necessary scientific information to support those measures and review the measures within a "reasonable period of time."

CONFLICT WITH INTERPRETATIONS
Regarding the SPS agreement: as the SPS is predicated on "science-based" risk assessment and strictly limits precautionary decision-making, it may come into conflict with biosafety instruments based on precautionary approaches, such as the CBD and CPB.

It was under the SPS agreement of the WTO that the United States (along with Canada and Argentina) challenged the EU de facto moratorium on GMO approvals. Similar challenges could arise for other biosafety legislation if it does not conform to SPS requirements. However, risk assessment standards included in the CPB may minimize future conflicts, given that these standards conform substantially to those foreseen by the SPS (Burgiel, 2002).

2.5.3 Intellectual property rights and access and benefit-sharing (ABS)

INTELLECTUAL PROPERTY RIGHT PROTECTION
TRIPS does not deal specifically with GMOs, but they fall under its purview where developers of a product seek intellectual property right protection (which is the case for nearly all commercialized GMOs). TRIPS requirements have definite potential to come into conflict with access and benefit-sharing (ABS) provisions in the ITPGRFA, the CBD, and the CPB.

TRIPS does not deal specifically with GMOs, but they fall under its purview where developers of a product seek intellectual property right protection (which is the case for nearly all commercialized GMOs). TRIPS requirements have definite potential to come into conflict with ABS provisions in the ITPGRFA, the CBD, and the CPB, all of which attempt to protect farmers' rights and prevent uncompensated use of traditional knowledge systems and biodiversity resources.

Recognizing and seeking to avoid the potential for conflicts, the Doha Ministerial (of the WTO) entrusted the Council for TRIPS a work programme to review, *inter alia*, the relationship between the TRIPS Agreement and the CBD, regarding the protection of traditional knowledge and folklore.

The International Union for the Protection of New Varieties of Plants (UPOV) shares goals with TRIPS and therefore faces similar potential conflicts. These goals are to encourage innovation and investment through protection of intellectual property rights.

ITPGRFA does not conflict with this goal, but seeks additional protection for the original human-biodiversity systems that generated products used in the development of patent-protected varieties, including through ABS provisions. The provisions of the three agreements, however, may be interpreted in mutually-compatible ways (Gerstetter *et al.*, 2007).

The CBD, like the ITPGRFA, seeks to implement ABS provisions that protect farmers and developers/conservers of traditional knowledge and biodiversity systems. The ITPGRFA specifically references the CBD, stating that "The objectives of this Treaty are the conservation and sustainable use of plant genetic resources for food

and agriculture and the fair and equitable sharing of the benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security [and that] These objectives will be attained by closely linking this Treaty to the Food and Agriculture Organization of the United Nations and to the Convention on Biological Diversity" (Part 1, Art. 1.1, 1.2). Again, depending on how TRIPS IPR and CBD ABS provisions are implemented, there may be conflicts over who enjoys intellectual property rights and what the responsibilities are for benefit sharing, but it is possible to interpret the rules to minimize conflict (Gerstetter *et al.*, 2007).

Table 2.1 | **Possible trade scenarios**

Country status *vis-à-vis* international agreements	**Status of trading partner**			
	Signatory to CPB and WTO	CPB, no WTO	WTO, no CPB	No WTO, no CPB
Signatory to CPB and WTO	Follow the norms of the Protocol and of WTO, attempt to minimize incompatibilities	Bilateral or regional accords compatible with the Protocol	Follow WTO norms, adopt bilateral or regional accords compatible with the Protocol	Bilateral or regional accords compatible with Protocol and WTO
CPB, no WTO	Follow WTO norms, adopt bilateral or regional accords compatible with the Protocol	Follow requirements of the Protocol	Bilateral or regional accords compatible with Protocol and WTO	Bilateral or regional accords compatible with the Protocol
WTO, no CPB	Follow WTO norms, adopt bilateral or regional accords compatible with the Protocol	Bilateral or regional accords compatible with Protocol and WTO	Follow WTO norms	Bilateral or regional accords compatible with WTO
No WTO, no CPB	Bilateral or regional accords compatible with Protocol and WTO	Bilateral or regional accords compatible with the Protocol	Bilateral or regional accords compatible with WTO	Compliance with the requirements of the importing country

Adapted from: Sarquis (2004).

POSSIBLE TRADE SCENARIOS
All possible trade scenarios and the country status *vis-à-vis* the discussed international agreements relating to trade are provided.

BOX 2.9

ACCESS AND BENEFIT-SHARING IN THE NAGOYA PROTOCOL

On 29 October, 2010, at its tenth meeting, and after six years of negotiations, the Conference of the Parties to the CBD adopted the *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity* (Nagoya Protocol). The Nagoya Protocol will enter into force 90 days after it has been ratified by at least 50 Parties.[10]

As noted in section 2.2.1 of this module, the CBD has three main objectives: (1) conservation of biological diversity, (2) the sustainable use of its components, and (3) the fair and equitable sharing of the benefits from the use of genetic resources. The Nagoya Protocol is "the instrument for the implementation of the access and benefit-sharing provisions of the Convention" (Article 4.4), and provides clarification on how to achieve the third objective.

The Nagoya Protocol is intended to provide legal certainty for both providers and users of genetic resources and associated traditional knowledge, and to ensure that providers of genetic resources receive a fair share of the benefits derived from their use (monetary and non-monetary). The Nagoya Protocol defines detailed and specific obligations to develop appropriate national legal frameworks governing access and benefit-sharing, and provides specifications on "prior informed consent" procedures,

10 Article 27(1) of the Nagoya Protocol: This Protocol shall enter into force on the ninetieth day after the date of deposit of the 50th instrument of ratification, acceptance, approval or accession by States or regional economic integrations that are Parties to the Convention.

"mutually agreed terms" and on access and benefit-sharing in relation to genetic resources and associated traditional knowledge held by indigenous and local communities (Articles 5, 6, 7, 8). It introduces a number of obligations to improve domestic legislation including effective dispute resolution and access to justice requirements in access contracts (Article 21), and by developing national model contractual clauses, codes of conduct, guidelines, and best practices (Articles 19, 20).

The Nagoya Protocol furthermore establishes obligations to comply with domestic access and benefit-sharing legislation of the Party that supplies the genetic resources and associated traditional knowledge (Articles 15, 16), including indigenous and local customary laws and procedures, in accordance with domestic law (Article 12).

The Nagoya Protocol lists a number of ways to facilitate its implementation, including through: capacity building, in particular for the least developed countries, small island developing States, transitional economies, and indigenous and local communities and stakeholders (Article 22); an ABS Clearing-House mechanism (Article 14); creation of national focal points and competent national authorities on access and benefit-sharing (Article 13); designation of checkpoints in relation to monitor the utilization of genetic resources (Article 17); technology transfer (Article 23); and awareness raising (Article 21).

2.5.4 Labelling issues related to international agreements

LABELLING ISSUES RELATED TO INTERNATIONAL AGREEMENTS
The main issue related to labelling arises over the different regulatory triggers for GMO regulation.

The main issue related to labelling arises over the different regulatory triggers for GMO regulation. Countries that consider specific, approved GMOs as products that are equivalent to their non-GM counterparts (the "product, not process" view) likewise question the need to label these products. This is particularly pertinent for regulations that require labelling for GM feed and other processed products that no longer contain GM material in the finished product.

Other countries, particularly those that use process as a trigger and take more precautionary positions (notably, the EU), claim that labelling is an important consumer information tool that is justified under the TBT agreement's authorization for non-discriminatory measures to achieve legitimate national objectives. It is possible that these differences will lead to a challenge at the WTO dispute settlement body.

In contrast to the WTO agreements, of which the effects on labelling are still unclear, the CPB has definite labelling requirements, as discussed in section 2.2.2. These do not affect national (or regional) labelling requirements, but do apply to internationally-traded LMOs intended for use as food, feed or for processing (but not processed foods containing GMOs). The CPB requirements originally required only a "may contain" label for shipments that could contain LMO-FFPs; since March 2006, however, any shipment containing LMO-FFPs identified through an identity preservation (IP) system must state the type of LMO and use a "does contain" label. For shipments where the contents are uncertain, the "may contain" label continues to apply (Gruère and Rao, 2007).[11]

11 Further descriptions of these requirements are available at http://www.cbd.int/biosafety/COP/MOP/result.aspx?id=8288, MOP BS-I/6, elaborating on CPB Article 18.

2.6 CONCLUSIONS: CHAPTER 2

Beyond the Cartagena Protocol on Biosafety, other international agreements, conventions and treaties, such as the WTO SPS and TBT Agreements and the Codex Alimentarius on food standards, governed by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations may impact directly or indirectly on the development of a national biosafety regulatory system. It is important that obligations under these agreements be considered when developing biosafety regulations, particularly for those countries that anticipate exporting GMOs. Where possible, attempts should be made to harmonize with risk assessment criteria and standards that have achieved international acceptance in either practice or principle.

CONCLUSIONS: CHAPTER 2
The discussed agreements may impact directly or indirectly on the development of a national biosafety regulatory system. It is important that obligations under these agreements be considered when developing biosafety regulations, particularly for those countries that anticipate exporting GMOs.

Table 2.2 | **Interactions among selected biosafety-related instruments**

Interactions	SPS	TBT	TRIPS	CBD	CPB	IPPC	Codex	Precautionary*	Product-based*
SPS	=	O	O	C	C	O	O	C	O
TBT	O	=	O	C	C	O	O	C/O	O
TRIPS	O	O	=	C	C	N/A	N/A	C	O
CBD	C	C	C	=	O	O	U	O	O,C
CPB	C	C	C	O	=	O	U	O	O,C
IPPC	O	O	O	O	O	=	U	U	O
Codex	O	O	N/A	U	U	U	=	O	O
Precautionary	C	C/O	C	O	O	U	O	=	C
"Product-based"	O	O	O	O,C	O,C	O	O	C	=

= the agreements are the same or completely compatible
O: the agreements are compatible, overlapping, or complementary
C: the agreements exhibit elements of conflict
U: unclear or not applicable
Some agreements may have both elements of compatibility and conflict. These are discussed further in the text.
* Precautionary refers to national and subnational frameworks that take a precautionary approach; "product-based" refers to those that take a product-based, or "science-based", approach.

INTERACTIONS AMONG SELECTED BIOSAFETY-RELATED INSTRUMENTS
A summary of all interactions between the discussed instruments, i.e. whether they are compatible, complementary or provide areas of conflict, is provided.

CHAPTER 3

CONSIDERATIONS OF LEGAL RELEVANCE TO DRAFTING NATIONAL FRAMEWORKS ON BIOSAFETY

CONSIDERATIONS OF LEGAL RELEVANCE TO DRAFTING NATIONAL FRAMEWORKS ON BIOSAFETY
This section discusses the relationship between international and national legal frameworks on biosafety and biotechnology, then addresses the intent and purpose of adopting national legal frameworks on biosafety.

The relationship between international and national legal frameworks dealing with biosafety and agricultural biotechnology is critical, as in most national legal frameworks it is through adoption into national regulatory frameworks that international agreements are put into practice. This section discusses that relationship, then addresses the intent and purpose of adopting national legal frameworks on biosafety. It next discusses the elements that countries must take into consideration when establishing their national biosafety frameworks, including the principles and approaches that they must consider, regulatory triggers for implementing legislation and approaches to addressing risk. Implementation of risk analysis, an important element of most legal frameworks on biosafety, as well as other available approaches to dealing with potential biosafety and other risks of biotechnology are then discussed.

The importance of transparency, communication, and public participation throughout the process is highlighted, along with monitoring and compliance requirements,

including the issue of liability and redress. Next the section addresses the issue of labelling, which has been an area of contention in international and state institutional relations. Finally, it covers issues of identity preservation, traceability, and monitoring.

3.1 RELATIONSHIP BETWEEN INTERNATIONAL AND NATIONAL BIOSAFETY FRAMEWORKS

RELATIONSHIP BETWEEN INTERNATIONAL AND NATIONAL BIOSAFETY FRAMEWORKS
Provisions of international instruments are most often not self-executing. International and national legal systems may require ratification by the parliament, and/or implementation through national legal instruments. This means that national legislation and regulations may be necessary to make agreements operational in national legal systems.

Provisions of international instruments are most often not self-executing. International and national legal systems may require ratification by the parliament, and/or implementation through national legal instruments. This means that national legislation and regulations may be necessary to make agreements operational in national legal systems. When existing national measures are insufficient, this may be done by amending existing measures or adopting new ones. Such measures should include all necessary elements to ensure appropriate implementation, including an administrative framework with appropriate decision-making powers.

States that are party to any international treaty are bound by that treaty and must comply with its obligations under the treaty. The party may itself decide on the legal, institutional and other means through which to achieve implementation. The tools generally used by states for this purpose are a national legal framework setting out rights and obligations for persons under its jurisdiction which aim at ensuring the implementation of the international instruments and an institutional framework to apply and enforce the national legislation (MacKenzie *et al.*, 2003).

Whether measures should be implemented through national laws or through regulations will depend on the internal law of the state concerned. Certain matters usually have to be dealt with by law, notably the establishment of offences and penalties. Others can be dealt with at the level of regulations issued by the relevant ministry or department that can be updated and amended more easily.

3.2 PURPOSES OF BIOSAFETY AND BIOTECHNOLOGY LEGISLATION

PURPOSES OF BIOSAFETY AND BIOTECHNOLOGY LEGISLATION
International and national biosafety frameworks, instruments, guidelines and regulatory systems reflect the need to protect human health and the environment from possible adverse effects of the products of modern (bio)technology.

International and national biosafety frameworks, instruments, guidelines and regulatory systems reflect the need to protect human health and the environment from possible adverse effects of the products of modern (bio)technology. Complex scientific, legal, social, environmental, health and economic issues have to be taken into account when developing or strengthening legal or regulatory frameworks for biosafety.

To understand the challenges of legal frameworks on biosafety, it is important to identify the interests and potential conflicts behind the areas that need to be covered. Institutions pass biosafety legislation to address biosafety specifically. They also pass biosafety and other biotechnology legislation to address a range of socio-economic issues that are important to their citizens. These include issues related to consumer protection, consumer information, labelling, trade, development, intellectual property rights, patenting, liability, ethical questions and food sovereignty. Some instruments attempt to address two or more of these issues (see Annex 2 for a chart listing the main issue areas addressed by different international agreements).

ISSUES ADDRESSED BY DIFFERENT INTERNATIONAL INSTRUMENTS
In relation to biosafety include environmental protection, human health and food safety, and consumer protection. Agreements also deal with public information, participation, and access to information and technologies.

Issues addressed by different international instruments which may relate to biosafety include environmental protection, human health and food safety and consumer protection. They also deal with public information, participation and access.

Many instruments serve more than one of these functions:

» **Environmental health and biodiversity:**
Instruments directly addressing these issues include IPPC, CBD, CPB, ITPGRFA. Indirectly affecting issues of environmental health and biodiversity are the Aarhus Convention, SPS, TBT.

» **Health and food safety:**
Codex Alimentarius, CPB, SPS, and TBT all directly address issues of human health and food safety.
The Aarhus Convention and CPD can be indirectly related to these and connected areas.

» **Consumer and citizen information and participation:**
Codex, the TBT, and the Aarhus Convention directly address consumer and citizen issues, while CBD, CPB and Aarhus all attempt to improve citizen information and participation provisions.

3.3 NATIONAL LEGISLATIVE FRAMEWORKS TO ADDRESS BIOSAFETY

Any biosafety regulatory system is based on the enabling legislation (acts, laws, decrees, and government orders) governing biosafety. At the national level, this derives from the authority to promulgate regulations, preempt subnational authorities, intercede in trade or domestic movements, and create enforcement agencies. The establishment of regulations (or executive orders) is necessary for enacting prohibitions, restrictions, permits and requirements under the authority of national legislation.

National regulatory frameworks also include guidelines and administrative procedures such as notification or information requirements. These policy instruments may be **mandatory or voluntary**. Voluntary instruments are generally easier and faster to adopt, and can be quite effective. However, in the absence of a binding legal instrument, the public may not have confidence that the government is adequately regulating products of biotechnology, or that developers are complying with voluntary guidelines.

NATIONAL LEGISLATIVE FRAMEWORKS TO ADDRESS BIOSAFETY
Any biosafety regulatory system is based on the enabling legislation (acts, laws, decrees, and government orders) governing biosafety. The establishment of regulations (or executive orders) is necessary for enacting prohibitions, restrictions, permits, and requirements under the authority of national legislation.

MANDATORY OR VOLUNTARY INSTRUMENTS
Voluntary instruments are generally easier and faster to adopt, and can be quite effective. However, in the absence of a binding legal instrument, the public may not have confidence that the government is adequately regulating products of biotechnology, or that developers are complying with voluntary guidelines.

National regulatory frameworks also need to address how notifications are handled. The CPB AIA requirements (see section 2.2.2; chart 1) should guide signatories to the CPB. Handling of notifications should, in any case, address information required in the notification (for example, name of the GMO/LMO), risk assessment (and determination of party or parties responsible for conducting the risk assessment), time frame for making decision, procedures for communicating decision and means for provision of public information and participation.

ELEMENTS OF NATIONAL BIOSAFETY LEGISLATION
Should include, as proposed by the UNEP Biosafety Toolkit: (1) biosafety policy providing an overarching framework and clear principles; (2) a regulatory regime; (3) means to address notifications or requests for authorizations; (4) means for enforcement and monitoring; and (5) public information, education and participation mechanisms.

Countries electing to develop national legislation on biosafety have different choices: (1) they can develop a framework act and implementing regulations to specifically address GMOs; or (2) they can review existing legal instruments, potentially introducing new provisions to regulate GMOs. The advantages of the former are specificity, flexibility and transparency. The disadvantages are the political difficulty and time required to adopt new legislation.

3.4 ELEMENTS OF NATIONAL BIOSAFETY LEGISLATION

Biosafety legislation at the national level should cover a number of elements. First, it should serve to implement the international binding agreements to which the country is signatory, and those elements of the non-binding agreements that the country has decided to implement. Countries choosing to regulate GMOs under the auspices of existing legal instruments should likewise determine that their existing legislation is in compliance with any international agreements to which they are signatories. Again, for most countries adopting legislation, the main agreements of interest will be the CBD/CPB and the WTO SPS and TBT agreements. Second, it must include all the national provisions necessary to foster or ensure implementation at national level.[1]

1 For a full discussion of national biosafety legislation implementation in accordance with the Cartagena Protocol, including case studies, see the United Nations Environment Programme's web site on biosafety at http://www.unep.org/biosafety/Default.aspx

The UNEP biosafety toolkit identifies five core components that every national biosafety framework should address: (1) biosafety policy providing an overarching framework and clear principles; (2) a regulatory regime; (3) means to address notifications or requests for authorizations; (4) means for enforcement and monitoring; and (5) public information, education and participation mechanisms.[2]

General operational principles to consider when creating biosafety frameworks include making the approach (1) preventative of harm; (2) responsive to unexpected events; (3) effective and efficient; (4) equitable; and (5) inclusive. Policies should be coherent and transparent. The remainder of this section addresses policies, principles, and components of biosafety frameworks, with a particular view to how national and international agreements relate and interact.

GENERAL OPERATIONAL PRINCIPLES
To consider when creating biosafety frameworks include making the approach
(1) preventative of harm;
(2) responsive to unexpected events;
(3) effective and efficient;
(4) equitable; and
(5) inclusive.

3.5 REGULATORY TRIGGERS

Determination of exactly what and how to regulate depends on the national policy on GMOs. Governments can consider GMOs as intrinsically novel, due to the techniques and process of their transformation, or as similar to other products of animal and plant breeding. Therefore, regulatory triggers can include either the product or the process by which it is developed.

REGULATORY TRIGGERS
Determination of exactly what and how to regulate depends on the national policy on GMOs; regulatory triggers can include either the product or the process by which it is developed.

It is generally acknowledged that product attributes define the associated risks, but many states and biosafety instruments utilize the process of genetic engineering as the de facto trigger for regulatory oversight. For example, the CPB addresses biosafety concerns that may be associated with the products of modern biotechnology, irrespective of the trait or traits that a GMO may express. Even some national frameworks based on the idea of "product, not process", such as the United States, include some elements of process-based regulation.

2 UNEP proposed format for preparation of a draft national biosafety framework, http://www.unep.org/biosafety/Toolkit.aspx

In the area of research on GMOs, where the final product attributes remain uncertain, international instruments and national biosafety frameworks include guidelines specifying levels of physical containment and health and safety procedures to be followed when undertaking research involving genetic manipulation. These usually include a system of mandatory notification and/or environmental risk assessment prior to the approval of experimental field trials, and standards for reproductive isolation and monitoring in order to minimize any impact on the environment or accidental release of genetically modified material.

3.6 RISK ANALYSIS

RISK ANALYSIS
Risk analysis is generally defined as a process comprising risk assessment, risk management and risk communication. Please refer to Module C for a detailed discussion of the process.

Risk analysis is generally defined as a process comprising risk assessment, risk management, and risk communication. Scientific risk assessment is the cornerstone of biosafety regulatory systems and public-policy decisions related to the safety and acceptability of GMOs. A strong scientific capacity and knowledge base is viewed as key to identifying hazards and assessing their impacts and likelihood of occurring. Nearly all of the international biosafety agreements discussed earlier highlight the importance of risk analysis; science-based risk assessment is recommended in the UNIDO Voluntary Code of Conduct, the WTO SPS, the Codex Statements of Principle Concerning the Role of Science in the Codex Decision-making Process, the CPB, and several FAO draft Codes of Conduct, among other agreements.

Risk assessment often addresses only biosafety issues strictly related to environmental and human health, leaving socio-economic, ethical and cultural issues to be addressed through other mechanisms. There may be cases where other factors are essential for making final decisions; these considerations are generally separated from the scientific risk assessment process, but may be considered during the risk management phase of risk analysis. This is the approach taken by the CPB, where socio-economic considerations are discussed in Article 26, separate from the articles addressing risk assessment.

BOX 3.1

STANDARDS FOR RISK ASSESSMENT

International agreements describe characteristics required for risk assessments to be considered adequate. Two important descriptions for standards for risk assessment are found in the WTO SPS and the Cartagena Protocol.

In the **SPS Agreement**, Article 5 specifies elements for consideration in risk assessment:

(2) In the assessment of risks, Members shall take into account available scientific evidence; relevant processes and production methods; relevant inspection, sampling and testing methods; prevalence of specific diseases or pests; existence of pest- or disease-free areas; relevant ecological and environmental conditions; and quarantine or other treatment.

(3) In assessing the risk to animal or plant life or health and determining the measure to be applied for achieving the appropriate level of sanitary or phytosanitary protection from such risk, Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing Member; and the relative cost-effectiveness of alternative approaches to limiting risks.

The **Cartagena Protocol on Biosafety**, Article 15(1) states: Risk assessments undertaken pursuant to this Protocol shall be carried out in a scientifically sound manner, in accordance with Annex III and taking into account recognized risk assessment techniques. Such risk assessments shall be based, at a minimum, on

STANDARDS FOR RISK ASSESSMENT International agreements describe characteristics required for risk assessments to be considered adequate. Two important descriptions for standards for risk assessment are found in the WTO SPS and the Cartagena Protocol.

information provided in accordance with Article 8 [on notification] and other available scientific evidence in order to identify and evaluate the possible adverse effects of living modified organisms on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

Annex III outlines the factors to be considered in risk assessments, and notes the following general principles:

3. Risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by, relevant international organizations.
4. Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.
5. Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.
6. Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the living modified organism concerned, its intended use and the likely potential receiving environment.

While risk evaluation is based on the available scientific data, risk management may also address other considerations such as social concerns or quantifiable economic impacts. Some international agreements, such as the CPB and the SPS, note economic concerns. In such cases, many institutions have attempted the creation of a regulatory structure that allows separation of the scientific risk assessment and regulatory decision-making processes to the extent possible. Such a **tiered approach** provides a system in which the regulatory decision is "informed," both by the scientific risk assessment and by other considerations.

TIERED APPROACH describes attempts to create a regulatory structure that allows separation of the scientific risk assessment and regulatory decision-making processes to the extent possible; the regulatory decision is "informed," both by the scientific risk assessment and by other considerations.

The drawback of this approach concerns the extent to which decisions may be subject to "political interference" or impinge on existing international trade agreements. Questions also remain about the possibility of separation of science and politics in practice. Adequate transparency, openness, and objectivity are key to the successful implementation of such an approach. Most biosafety frameworks do not attempt to include broader **socio-economic considerations** (excluding economic consequences) into the process for individual product approvals. These important considerations are instead dealt with by establishing expert bodies responsible for providing governments with policy advice on ethical, legal, or social issues related to the adoption of new technologies. The exploration of these issues can serve both to develop a public consensus on the acceptability of various technologies and to guide the evolution of a policy framework for regulation.

SOCIO-ECONOMIC CONSIDERATIONS are excluded from most biosafety frameworks; instead, these important considerations are dealt with by establishing expert bodies responsible for providing governments with policy advice on ethical, legal, or social issues related to the adoption of new technologies.

In tiered systems, it is generally the risk management phase of risk analysis that provides an opportunity to consider some of these issues. The underlying principle of risk management is to identify and take steps to eliminate or minimize to an acceptable level any risks identified in the risk assessment. Risk management strategies vary with circumstances and can embrace a number of techniques ranging from an outright ban to softer approaches that might include educating users of the proper application of an end product. In particular, post-approval monitoring, labelling and traceability can be used within risk management strategies and are described below.

As is the case with risk assessment, additional principles have been recognized by the international community that provide a framework for the application of risk management, especially as it relates to international trade. The need for risk management measures to be "necessary" and where implemented, "proportional" to the risks identified are two principles that share the widest recognition at the international level. Calls for necessity and proportionality are common to both biosafety and food safety instruments (Glowka, 2003). Among others, the WTO SPS and TBT Agreements require that risk management measures be non-discriminatory, necessary, proportional, and justified.

Risk communication has developed from a one-way, post-decision process to a multi-party, iterative process that occurs throughout the stages of risk analysis. It is closely related to efforts to increase public awareness and knowledge and to enhance public participation. Several international agreements related to biosafety contain specific references to risk communication as part of the risk analysis process (Box 3.2).

3.6.1 Approaches to risk analysis

APPROACHES TO RISK ANALYSIS
Different frameworks on biosafety approach the question from different perspectives; please also refer to *Module C for further information.

Different frameworks on biosafety approach the question from different perspectives. Some take the position that there is no special novelty associated with GMOs, particularly in cases where there is *familiarity* with the host and recipient organisms. In such cases, they hold that there should be an assumption of *substantial equivalence* unless the product itself exhibits unexpected characteristics, and that, concomitantly, no additional information provision is warranted unless there are questions related to allergenicity or public health (as with crops or animals altered to produce pharmaceutical products, for example).

BOX 3.2

RISK COMMUNICATION

From **Codex Alimentarius**, Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44-2003) (amended 2008, www.codexalimentarius.net/download/standards/10007/CXG_044e.pdf):

22. Effective risk communication is essential at all phases of risk assessment and risk management. It is an interactive process involving all interested parties, including government, industry, academia, media and consumers.

23. Risk communication should include transparent safety assessment and risk management decision-making processes. These processes should be fully documented at all stages and open to public scrutiny, whilst respecting legitimate concerns to safeguard the confidentiality of commercial and industrial information. In particular, reports prepared on the safety assessments and other aspects of the decision-making process should be made available to all interested parties.

24. Effective risk communication should include responsive consultation processes. Consultation processes should be interactive. The views of all interested parties should be sought and relevant food safety and nutritional issues that are raised during consultation should be addressed during the risk analysis process.

RISK COMMUNICATION
According to the Codex Alimentarius: Effective risk communication is essential at all phases of risk assessment and risk management. It is an interactive process involving all interested parties, including government, industry, academia, media and consumers.

This position is generally associated with an approach that follows conventional risk assessment, with scientific risk assessment addressing biosafety issues exclusively, followed by risk management to determine how to address issues raised during risk assessment. This approach leads to the view that there is no reason to restrict trade in GMOs unless particular risk characteristics have been identified.

By contrast, other institutions base their approach on the novelty of the process of genetic modification, and use the concept of "substantial equivalence," if at all, as a tool in the risk analysis process. Instead, they prioritize *precaution* and *prevention* of risk. Many seek to incorporate concerns beyond those that could be defined strictly as biosafety (that is, risks to human and environmental health). Economic concerns include not only those about trade restriction, but also concerns about potential economic damage. Other socio-economic concerns are also considered, including traditional livelihoods, food security and food sovereignty.

3.6.1.1 Familiarity

FAMILIARITY
Most genetically modified organisms to date have been developed from organisms that are "familiar", i.e. there is substantial available information about the organism's attributes, and long history and experience of its safe use.

Risk assessment of GMOs requires information on the identity, characteristics and history of safe use of the organism that is subjected to genetic modification. Most GMOs to date have been developed from organisms that are "familiar", i.e. there is substantial available information about the organism's attributes, and long history and experience of its safe use. Both Agenda 21, Chapter 16 and the UNEP Guidelines use familiarity as a basis for conducting risk assessments.

The concept of familiarity provides a way to recognize the potential risks by using already available information on the attributes of the organisms involved in the transformation. Familiarity can help devise effective methods to avoid or manage the risks to acceptable levels. For example, it may be possible to determine the potential for invasiveness of the GM crop based on knowledge of its ecological characteristics (e.g. presence of traits that are associated with invasiveness) and

the presence of wild compatible relatives. Likewise, it may be possible to identify the potential allergenicity of the GMO if knowledge and history of safe use of the origin/source of the gene used in genetic modification is available. In this context, the concept of familiarity is not a risk assessment by itself but can be a useful tool for identifying, evaluating and managing risks.

Familiarity, however, has its drawbacks as a risk analysis tool. Many ecologists question its usefulness, believe that it is an intrinsically subjective concept, and caution that it can lead to false reliance on previous knowledge that may not apply in a given situation (e.g. Marvier and Kareiva, 1999; Antonovics, 1999). Furthermore, the depth of familiarity with a crop is often more geared to its agronomic performance than to potential environmental impacts (Gaugitsch, 2002). While its usefulness as evidence is contested, the concept of familiarity may be more useful as a benchmark or comparator, and in identifying areas where there is inadequate knowledge of the characteristics of the organism involved (see e.g. Kareiva and Marvier, 2000; Kapuscinski & Hallerman, 1995). Critiques of the principle of familiarity highlight the importance of post-commercial monitoring to confirm pre-planting assumptions based on familiarity.

SUBSTANTIAL EQUIVALENCE
Recognized as one of the principles for environmental risk assessment. According to CBD: Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.

3.6.1.2 Substantial Equivalence

Internationally, the concept of substantial equivalence is recognized as one of the principles for environmental risk assessment by the CPB, and in food safety assessment by the Codex Alimentarius Commission. The relevant texts (italics provided) are as follows:

Cartagena Protocol on Biosafety (2000)

Annex III 5 – Risk Assessment

Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing

detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, *should be considered in the context of the risks posed by the non-modified recipients or parental organisms* in the likely potential receiving environment.

Codex Alimentarius Commission Principles and Guidelines on Foods Derived from Biotechnology (2003)

Section 3, Principles, Article10 – Risk Assessment

Risk assessment includes a safety assessment (…) The safety assessment *should include a comparison between the food derived from modern biotechnology and its conventional counterpart focusing on determination of similarities and differences*. If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterized to determine its relevance to human health.

As an approach, it should be noted that the concept of substantial equivalence is considered a *starting* point for the safety assessment to structure the safety assessment procedure, and focus on the identified differences that may require further testing. Its application is limited by the choice of an appropriate comparator and availability of sufficient scientific information relevant to the risk assessment.

These points are illustrated in the three cases presented below.

- *GMOs that are shown to be substantially equivalent to the conventional counterparts* may be regarded as being "as safe as" their counterpart. No further safety considerations other than those for the counterpart are necessary.
- *GMOs that are substantially equivalent to the conventional counterpart except for defined differences* need further safety assessment that should focus only on the defined differences. Typically, the defined differences will result from the intended effect of the genetic modification that may, or may not, change the endogenous traits, or produce new traits in the host organism.

» *GMOs that are not substantially equivalent to the conventional counterpart*. In these cases, the concept of substantial equivalency cannot be applied.

The proper application of familiarity and substantial equivalence, in particular the assumptions upon which both principles are founded and applied, is an outstanding issue that may determine the extent to which the risks of GMOs can be accurately identified and subsequently minimized or eliminated. In particular, some uses of substantial equivalence are becoming increasingly criticized.

The concept of substantial equivalence has undergone major reassessment. Initially, it was thought that if a genetically modified food was "substantially equivalent" to its traditional counterpart, a risk assessment would not be necessary. Comparisons focused on attributes such as protein, carbohydrate and fatty acid levels between the novel food and its traditional counterpart. However, there were no clear and universal guidelines stipulating what to test and how similar the items in question should be. It has been said that the amount of comparative data required to establish "substantial equivalence" involved "a somewhat subjective judgment" (Royal Society, 2002).

The approach proved immensely controversial. Consumer organizations, environmental groups and a few leading scientists criticized "substantial equivalence" for helping to play down the novelty of genetic engineering and facilitating its commercialization. Over the years, the approach has come to mean something very different and it has ultimately been demoted in the regulatory framework - albeit implicitly (Royal Society, 2002).

Applying the concept of substantial equivalence requires that sufficient analytical data be available in the literature, or be generated through experimentation, to allow effective comparison between the novel plant and its traditional counterpart. A problem arises in that risk factors have generally not been established for traditionally bred plant varieties and so there is very little baseline information

about the environmental risks associated with their introduction. This suggests a **basic limitation of the substantial equivalence concept**: dependence on a comparator (base product), and on the information that is available or can be generated for the comparator, means safety assurance is relative to the components assessed for the particular comparator. The choice of comparator is therefore crucial to effective application of the concept of substantial equivalence.

BASIC LIMITATION OF THE SUBSTANTIAL EQUIVALENCE CONCEPT
Applying the concept of substantial equivalence requires that sufficient analytical data be available in the literature, or be generated through experimentation, to allow effective comparison between the novel plant and its traditional counterpart. A problem arises in that risk factors have generally not been established for traditionally bred plant varieties and so there is very little baseline information about the environmental risks associated with their introduction.

3.6.1.3 Precaution

Precaution is an approach related to decision-making in situations of scientific uncertainty. Precaution is particularly relevant to GMO issues because of the inherent scientific uncertainty and difficulties of predicting potential impacts. The precautionary approach allows decision-makers to take account of scientific uncertainty and to make judgments based on limited scientific evidence and available knowledge as to the level of acceptable uncertainty in a given context. Environmental measures based on precaution should be proportionate to the anticipated risk and non-discriminatory.

Principle 15 of the Rio Declaration (Agenda 21) states that "lack of full scientific certainty shall not be used as a reason for postponing cost effective measures to prevent environmental degradation."

The CPB reaffirms in its preamble the **precautionary approach** contained in Principle 15 of the Rio Declaration on Environment and Development, stating lack of certainty "shall not be used as a reason to postpone measures to avoid or minimize a threat of significant reduction or loss of biodiversity." The precautionary approach is also referred to in Article 10.

Under the Protocol, decisions of the contracting party importing a GMO destined for first-time release into the environment (and where necessary for GMOs intended for direct use as food or feed, or for processing) must be according to a risk assessment.

BOX 3.3

PRECAUTIONARY APPROACHES

Definitions of precaution, or descriptions of precautionary approaches, exist in several international agreements.

Principle 15 of the 1992 **Rio Declaration** on Environment and Development defines the precautionary approach as follows: "*In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.*"

In the **Convention on Biological Diversity**, the Preamble does not specifically refer to "precaution," but states that "*...where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat.*"

The **Cartagena Protocol**, in turn, specifically references Principle 15 of the Rio Declaration in its preamble and refers to precaution in several other sections, such as: Article 1, indicating that the objective of the Protocol is "*in accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on environment and Development*"; Article 10.6 and 11.8, stating: "*Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of an LMO on biodiversity, taking into account risks to human health, shall not prevent a Party of import from taking a decision, as appropriate, with regard to the import of the LMO in question, in order to avoid or minimize such potential adverse effects*"; and Annex III on risk assessment, stating: "*Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a*

PRECAUTION
Precaution is an approach related to decision-making in situations of scientific uncertainty. Precaution is particularly relevant to GMO issues because of the inherent scientific uncertainty and difficulties of predicting potential impacts.

THE PRECAUTIONARY APPROACH
According to the Rio Declaration: "lack of full scientific certainty shall not be used as a reason for postponing cost effective measures to prevent environmental degradation."

particular level of risk, an absence of risk, or an acceptable risk" (http://www.cbd.int/biosafety/articles.shtml?lg=0&a=cpb-10).

The **SPS** agreement Article 5(7) permits the taking of provisional measures when there is insufficient scientific evidence to permit a final decision on the safety of a product of process:
"*In cases where relevant scientific evidence is insufficient, a Member may provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information, including that from the relevant international organizations as well as from sanitary or phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time.*"

Regional agreements, too, make mention of precaution. Notable among them is the **European Union**'s description of the precautionary principle, as mentioned in the EC Treaty (article 174) and presented in the European Commission's Communication on the Precautionary Principle, COM (2000)1, available at: http://ec.europa.eu/environment/docum/20001_en.htm. The Communication specifies that:

"*Recourse to the precautionary principle presupposes that potentially dangerous effects deriving from a phenomenon, product or process have been identified, and that scientific evaluation does not allow the risk to be determined with sufficient certainty. The implementation of an approach based on the precautionary principle should start with a scientific evaluation, as complete as possible, and where possible, identifying at each stage the degree of scientific uncertainty.*"

In the framework of food safety, the precautionary principle has been recognized in Article 7 of Regulation 178/2002 on the principles of food safety legislation (OJL 31 of 1.2.2002).

However, lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of potential adverse effects should not prevent the contracting party of import from taking a decision, as appropriate, in order to avoid or minimize potential adverse effects. Parties may take into account the precautionary approach in reaching decisions on imports of LMO-FFPs (Art. 11[8]).

In the food safety area, it appears the Codex Commission is embracing a precautionary approach, even if the term is not explicitly referred to in the Codex itself. For example, the Codex Proposed Draft Principles for the Risk Analysis of Foods Derived from Modern Biotechnology state that risk managers are to account for the uncertainties identified in the risk assessment and manage the uncertainties (Sec.3 [18]).

In the area of trade, the WTO SPS Article 5.7 of the SPS Agreement permits the taking of provisional measures when there is insufficient scientific evidence to permit a final decision on the safety of a product or process. In such cases, measures can be adopted on the basis of the available pertinent information about the health risk(s) of a product or process. However, when taking such a provisional measure, a Member must seek the additional information necessary for a more objective assessment of the risk(s), and review the SPS measure within a reasonable period of time. It should be emphasized that Article 5.7 is a "qualified exemption" in that the following four conditions must all be met for the provision to be legitimately invoked: (i) an Article 5.7 SPS measure may be imposed only in a situation where relevant scientific information is insufficient; (ii) the provisional measure must be adopted on the basis of available pertinent information; (iii) the Member adopting the measure must seek to obtain the additional information necessary for a more objective assessment of risk; and (iv) the Member must review the SPS measure within a reasonable period of time.

BOX 3.4

WTO DS26, THE BEEF HORMONES DISPUTE

The "beef hormone case" is a good example of potential conflicts between precautionary policies and trade agreements. The EU in 1985 adopted policies against using growth hormones in cattle production (EC Directives 81/602, 85/358, 96/22), on the basis of health and consumer concerns. The directives also led to the EU banning imports of meat produced using growth hormones. In 1997, the United States and Canada filed a WTO complaint against the EU for its import ban, saying that it had no scientific basis. While the SPS Agreement allows for Members to adopt more stringent policies than the agreed international standards, it has the concomitant requirement that any such policies be justified by risk assessment.

As discussed in sections 2.2.3 and 3.6, the risk assessment process is meant to avoid, insofar as possible, the inclusion of concerns beyond direct human and environmental health hazards. Therefore, consumer opinion, along with other socio-economic and/or ethical concerns, is excluded from consideration. In this case, the EU had in fact conducted a risk assessment that did not back a ban, as it showed no significant impact on human health from growth hormones in beef.

While some supporters of the ban argued that the scientific studies were inadequate, the EU itself did not argue the case on the basis of the risk assessment they had conducted, and instead marshalled arguments based on consumer perception and trust. They claimed that, due to a series of public health scandals (primary among them BSE ["mad cow" disease]), the ban was necessary to respond to public concerns.

While this position may have had policy merit, it did not convince the arbiters, who were constrained to deciding the case on the basis of the risk assessment *per se*, as

foreseen in the SPS agreement. The WTO trade dispute resolution panel, and subsequently the WTO's appellate body, found that the EU's basis for the ban was not justifiable. The trade dispute panel noted three main problems: (1) other international standards did not back up the ban; (2) the policy was not consistent; and (3) the EU's decision was not based on findings of a risk assessment, as required by the SPS agreement. The appellate decision demurred from the panel's decision in the areas of harmonization and discrimination, but noted, in particular, that the risk assessment was too broad and did not adequately show that the EU's policies achieved additional health protection.

While this decision seems to indicate an anti-precautionary stance, it is not definite what effect it will have on future decisions. Precautionary policies rarely call for "absolute certainty" of no harm, and the WTO's decision in this case is more tied to the fact that the EU was unable to persuade the appellate body that their standards did a better job of protecting human health than the Codex standards. Furthermore, the appellate body's decision can be read (and, indeed, has been read thus by the EU) as confirming that the EU did have the right to set more stringent standards than the Codex.

Adapted from: Giandomenico, 2002 and Holmes, 2006.

3.6.1.4 Prevention

PREVENTION
The duty to take preventive measures is laid down by most international environmental instruments. Prevention is more cost effective and environmentally desirable than remedial measures taken after damage to the environment and human and animal life.

The duty to take preventive measures is laid down by most international environmental instruments. Prevention is more cost effective and environmentally desirable than remedial measures taken after damage to the environment and human and animal life. At times destruction, eradication or other control measures may be impossible and the ecological damage irreversible (Shine, Williams and Gundling, 2000).

In general terms, prevention applies to activities that may have serious adverse effects on the environment. It does not impose an absolute duty on states to prevent all harm but requires them to exercise due diligence and act reasonably and in good faith in prohibiting or regulating activities that could have such results. Governments should also put measures in place to prevent or minimize damaging consequences of activities that are permitted.

Many international and national instruments establish a threshold above which preventive measures should be taken. This is true for biosafety measures, as well. Preventive actions must be different for intentional and unintentional movement of GMOs or their release into the environment. For intentional unauthorized movements or release, prevention may take the form of total prohibition or partial prohibition usually under a permit to which conditions may be attached. For unintentional release, the likelihood of GMOs escaping should be prevented.

The CPB in Article 2 states as its objective "to ensure that the development, handling, transport, use, transfer and release of any LMOs are undertaken in a manner that prevents or reduces the risks to biological diversity, taking also into account risks to human health." It emphasizes that legal rules should be designed to prevent damage from occurring rather than attempting to remedy damage after it has occurred. Article 2(2) provides that parties should be guided by the preventive approach in relation to the following activities involving LMOs: development, handling, transport, use, transfer and release.

ADAPTIVE MANAGEMENT Is a technique that can augment traditional risk management by taking into account new information.

3.6.1.5 Adaptive management

Adaptive management is a technique that can augment traditional risk management by taking into account new information. It involves adjusting management in light of experience and additional data, and essentially means "learning by doing." It

is an especially valuable technique for new technologies and new applications of existing technology, as these often involve uncertainties and issues that require reassessment based on experience. As such, it can be a valuable tool in biosafety management. Indeed, it is a principle that is being incorporated into biosafety capacity development training on the CBD by UNEP (see, for example, http://www.cbd.int/doc/meetings/nbsap/nbsapcbw-pac-01/official/nbsapcbw-pac-01-01-add1-en.pdf).

3.7 PUBLIC PARTICIPATION AND ACCESS TO INFORMATION

PUBLIC PARTICIPATION AND ACCESS TO INFORMATION
Many international instruments mandate public participation in environmental planning and decision-making, which is increasingly reflected in national legal systems and administrative procedures.

Many international instruments mandate public participation in environmental planning and decision-making, which is increasingly reflected in national legal systems and administrative procedures. Participatory approaches need to be complemented by judicial review procedures to guarantee individual rights. Affected parties should be given the right to appeal decisions for the refusal of permits. On the other hand, there should be judicial remedies available for interested individuals or groups to challenge administrative decisions on GMO imports, exports or activities that are considered to be unlawful or inconsistent with the protection or conservation objectives of relevant legislation.

One of the most useful legal tools for realizing the potential and avoiding the risks of modern biotechnology may be legally requiring public participation in the policy-making and regulatory decision-making processes. Opening decision-making processes up to the public helps to ensure that decision-makers have the best information at their disposal in order to evaluate the benefits and risks that modern biotechnology could present. Public participation can also help to ensure better transparency and accountability in decision-making (see Box 3.5: Public participation mechanisms).

Access to accurate information related to biotechnology in general and GMOs in particular is a cornerstone of any system to realize modern biotechnology's benefits and avoid its risks. The accessible information can include permit applications, environmental and other assessment results, the results of consultations with the public, as well as information on approvals and denials (Glowka, 2003). Access to information is especially important because GMO releases generally take place on a case-by-case basis.

A sub-area of access to information is the extent to which a permit applicant may withhold confidential information and prevent its dissemination to the public during the regulatory review and decision-making process. The possibility to withhold commercially sensitive information is an established principle at international and national levels (Glowka, 2003). The issue of CBI is also discussed in Section 2.5.3 on the relationship between IPR and access and benefit-sharing..

PUBLIC PARTICIPATION MECHANISMS
Apart from new rules to increase openness, transparency, and information sharing with the public, governments worldwide have also sought to improve governance by making the process of decision-making itself more democratic.

BOX 3.5

PUBLIC PARTICIPATION MECHANISMS

Public participation in environmental policy-making has been an increasingly important concern for governments. Apart from new rules to increase openness, transparency and information sharing with the public, governments worldwide have also sought to improve governance by making the process of decision-making itself more democratic.

Several new institutions and techniques form part of this scheme to solicit information and public input. Initially used principally in Northern Europe and the United States (citizen juries, for example, originated in Germany and the United States, while consensus conferences were first promoted in Denmark), these techniques are spreading globally.

Mechanisms include citizen juries, expert committees (with or without public or lay members), public oversight boards, polls, consensus conferences, focus groups, participatory foresight exercises and public hearings. Many of these techniques are focused on gaining the viewpoint of non-specialists (as opposed to expert committees).

These techniques aim for inclusiveness and representativeness as well as to provide mechanisms for information provision, discussion and debate. They are intended to give policy-makers a sense of the will of the citizenry, as well as an understanding of the factors citizens consider when reaching decisions.

Governments are also exploring improving access to upstream decision-making by including civil society organizations and citizen representatives on science panels (for example, the European Union has several initiatives exploring the role of civil society in science policy).

Citizen juries and consensus conferences have been used to solicit public input in a number of areas of environmental decision-making, from park management to water resources to food and agriculture, and in particular, in areas that have engendered substantial controversy, such as agricultural biotechnology. The techniques have been used in the United States, Europe, and developing countries, such as India and Brazil. One of the most recent high-profile examples was as part of a broad-based effort by the British Government to involve the public in policy decisions on genetically modified (GM) organisms, GM Nation, in 2003. Other examples include citizen juries in Brazil (2001) and consensus conferences in Belgium (2003), Japan (2000), Australia (1999), Argentina (2000), and India (2001).

3.8 TRANSPARENCY OF DECISIONS AND PUBLIC ENGAGEMENT

TRANSPARENCY OF DECISIONS AND PUBLIC ENGAGEMENT
Transparency refers to the amount and level of information that governments provide on why and how certain products are regulated, on how risk assessments are performed and decisions made, and on what conclusions are reached.

The twin issues of public information and participation relate to the degree of transparency in a regulatory system and to the extent to which the public can provide input to the formulation either of a regulatory policy, or of specific regulatory decisions. In this context, transparency refers to the amount and level of information that governments provide on why and how certain products are regulated, on how risk assessments are performed and decisions made, and on what conclusions are reached. Transparency can also relate to the perceived independence and objectivity of the regulatory decision-makers. Although closely related, public information and participation have some mutual exclusivity, as it is certainly possible to have an open and transparent process that, however, does not involve public input. Greater transparency concerning both the risks and benefits of biotechnology products and government decision-making is an essential component of building public trust in new technologies. The dissemination of more and better information on agricultural biotechnology is a stabilizing force because, while the public may not generally read scientific studies, risk assessments, or government decision documents, opinion leaders, members of special interest groups, or others who hope to shape public opinion, do (McLean *et al.*, 2002).

GOVERNMENT POLICY ON TRANSPARENCY
Will determine the extent to which the public and special interest groups will contribute to the development of a national biosafety policy; the opportunities for public participation in the risk-assessment and decision-making process; and the degree to which the public will have ready access to information about the biosafety system.

Government policy on transparency will determine the extent to which the public and special interest groups will contribute to the development of a national biosafety policy; the opportunities for public participation in the risk-assessment and decision-making process; and the degree to which the public will have ready access to information about the biosafety system. Ideally, the process used to develop a national biosafety system should be transparent and the level of involvement of the public and/or stakeholder or special interest groups as legislation, regulations, or guidelines are being developed, as well as after they have been adopted, ought to be considered.

As a minimum, the process and criteria for risk assessment and risk management should be widely published so that developers, stakeholders and the public can trust the biosafety system to be both credible and predictable. Some jurisdictions have surpassed this level: they additionally notify the public when applications for the environmental safety assessment of a GMO are received by the competent authorities, and also when a regulatory decision is made. Within the context of implementing a biosafety system, opportunities for public engagement may be provided through formalized requests for public input. Most commonly, the public is provided with an opportunity to evaluate summary information about the GMO under review and to submit comments in this regard.

3.9 MONITORING[3] AND COMPLIANCE

MONITORING AND COMPLIANCE
Two types of monitoring are considered: monitoring of obligations under different international agreements and related compliance, and post-release monitoring, namely a systematic process of monitoring or surveillance of GMOs after release into the market or the receiving environment.

There are two types of monitoring that are important for biosafety. First, there is monitoring of obligations under different international agreements and related compliance. The CPB, notably, has a monitoring and reporting requirement. Article 33 of the CPB addresses monitoring and reporting of obligations under the Protocol, requiring reporting of what steps Members have taken to implement the Protocol.

Second, there is post-release monitoring, namely a systematic process of monitoring or surveillance of GMOs after release into the market or the receiving environment. Many countries recognize the need for a long-term monitoring of the cumulative effects of GMOs but to date few have implemented such a system.

With respect to monitoring and compliance, the Biosafety Clearing-House (BCH), a mechanism set up by the CPB, facilitates exchange of information about transboundary movements of GMOs. Other international and national organizations also disseminate information from research on GMOs that can be useful in developing

3 See Module D of this Compendium for a more detailed discussion of pre- and post-release monitoring.

monitoring plans; these include the International Centre for Genetic Engineering and Biotechnology (ICGEB), FAO, WHO, Codex, the OECD, and national agencies such as the United States Department of Agriculture (USDA). There remain practical, technical and economic limitations to monitoring for GMOs to ensure that national and international rules and regulations are respected. Given these difficulties, ensuring compliance remains difficult. Several environmental non-governmental organizations (NGOs) have focused their efforts in this area, alerting their Members and national governments to contamination incidents. Effective monitoring could assist in minimizing these events.

3.10 LIABILITY AND REDRESS

LIABILITY AND REDRESS
Another aspect of biosafety regulation that is related to monitoring and compliance is liability. International agreements mention the issue of liability, but as of March 2009, none contain binding provisions.

Another aspect of biosafety regulation that is related to monitoring and compliance is liability. Initial CBD discussions raised the issue, but the parties did not agree on a set of requirements for liability and redress. Other international agreements mention the issue of liability, but do not contain binding provisions. As a result, Article 27 directed the COP to "adopt a process with respect to the appropriate elaboration of international rules and procedures in the field of liability and redress for damage resulting from transboundary movement of LMOs, analyzing and taking due account of the ongoing process in international law on these matters, and shall endeavour to complete this process within four years."

On 15 October, 2010, at its fifth meeting, the Conference of the Parties serving as the meeting of the Parties to the Protocol (COP-MOP, the governing body of the Cartagena Protocol on Biosafety to the CBD) adopted the *Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety* (N-KL Supplementary Protocol). The N-KL Supplementary Protocol will enter into force 90 days after it has been ratified by at least 40 Parties.[4]

4 Article 18.1 of the Nagoya-Kuala Lumpur Supplementary Protocol: This Supplementary Protocol shall enter into force on the ninetieth day after the date of deposit of the fortieth instrument of ratification, acceptance, approval or accession by States or regional economic integration organizations that are Parties to the Protocol.

As noted in section 2.2.1 of this module, the CBD has three main objectives: (1) conservation of biological diversity, (2) the sustainable use of its components, and (3) the fair and equitable sharing of the benefits from the use of genetic resources. The N-KL Supplementary Protocol addresses issues relating to conservation of biological diversity, the first objective – many Parties to the Cartagena Protocol felt that the Protocol needed specific rules addressing liability and redress. In response, the N-KL Supplementary Protocol elaborates international rules and procedures in the field of liability and redress for damage resulting from transboundary movements of living modified organisms.

DAMAGE
Defining damage is important for liability approaches; a proposed definition is "adverse or negative effect on biological diversity" that must be measurable and significant.

In particular, the N-KL Supplementary Protocol defines what constitutes "damage to biodiversity" (traditional damage, such as personal injury, loss or damage to property or economic interests, is not covered by the N-KL Supplementary Protocol). "**Damage**" is defined as a measurable and significant "adverse effect on the conservation and sustainable use of biological diversity, taking also into account risks to human health..." (Articles 2.2[b] and 2.3).

Once a determination is made that measurable and significant damage resulting from transboundary movement of LMOs exists (and, per Article 4, a causal link is made between the LMO in question and the damage) the N-KL Supplementary Protocol has adopted an administrative approach for addressing such damage. Signatories to the Supplementary Protocol are required to adopt response measures in the case of damage (and implement them through domestic law [Article 12]), including (1) identification of the operator who caused the damage; (2) evaluation of the damage; and (3) response measures to be taken by the operator (Article 5.2). In case of failure by the operator to respond in a timely fashion, the competent authority itself may take action (Article 5.4) and recover costs of appropriate response from the operator (Article 5.5). Response measures include actions to (1) prevent, minimize, contain, mitigate, or otherwise avoid damage, as appropriate; and (2) restore biological diversity (Article 2.2[d]).

As noted in the Nagoya-Kuala Lumpur Supplemental Protocol, liability and redress provisions require showing a causal link between damage and the activity. Further considerations are valuation of damage; means of assigning liability to the parties involved; compensation; and finally, response measures in the case of damage could include minimization, containment, restoration, and/ or replacement of biodiversity losses. Additional considerations for liability and redress legislation would include compensation, cases for exemptions or mitigating factors, and the idea of insurance coverage for operators.

DOMESTIC LIABILITY AND REDRESS REGIMES
An example being Article 14 of the African Model Law on Safety in Biotechnology, which suggests an extensive list of elements for a liability and redress regime that should be incorporated into domestic biosafety legislation.

In addition to action via international instruments such as the CBD, countries may develop **domestic liability and redress regimes** or use existing civil law remedies where these are appropriate and adequate. Some models for this exist, including Article 14 of the African Model Law on Safety in Biotechnology, which suggests an extensive list of elements for a liability and redress regime that should be incorporated into domestic biosafety legislation. It makes any person who imports, arranges transit, makes contained use of, releases or places on the market a GMO or GMO product strictly liable for any harm caused by the GMO or product. The harm must be fully compensated.

COMPENSATION
In the case of harm to the environment, or biological diversity, compensation should include the costs of reinstatement, rehabilitation or clean up measures incurred as well as the costs of preventive measures.

Liability also extends to the person responsible for any activity that results in damage, injury or loss, as well as to the provider, supplier or developer of the GMO or GMO product. Liability can be joint or several (Art. 14 [2] and [3]. Where harm occurs to the environment or biological diversity, **compensation** should include the costs of reinstatement, rehabilitation or clean up measures incurred as well as the costs of preventive measures (14[4]). In case of harm to human health, compensation should include costs of seeking and obtaining treatment, compensation for disability or diminished quality of life, and costs of reinstating quality of life, and compensation for loss of life and related expenses (14[5]).

Liability further extends to harm or damage caused directly or indirectly by the GMO or GMO product to economic, social or cultural conditions, including negative impacts on the livelihood or indigenous knowledge systems or technologies of a local community. Liability also extends to any damage or destruction arising from incidence of public disorder triggered by the GMO or GMO product, any disruption or damage to production or agricultural systems, reduction in yields, soil contamination, damage to biological diversity, the economy of an area or community and any other consequential damage (14[6]).

3.11 BASIC ASPECTS OF LABELLING[5]

BASIC ASPECTS OF LABELLING
The labelling of GMOs or products derived from GMOs is a sub-area of access to information with three main uses of labelling in consumer protection and consumer and environmental safety: (1) consumer right-to-know concerns; (2) protection from misleading claims; and (3) consumer education on issues related to human and environmental health.

The labelling of GMOs or products derived from GMOs is a sub-area of access to information. Glowka (2003) provides a good overview of three main uses of labelling in consumer protection and consumer and environmental safety: (1) consumer right-to-know concerns; (2) protection from misleading claims; and (3) consumer education on issues related to human and environmental health. Labelling is being considered, and in some cases is already being used, in the biosafety and food safety areas in order to provide consumers with information on the GMO or GMO-derived product that they are either considering purchasing or are already using.

One aspect of labelling is premised on the principle that the consumer has a right to know what he or she is purchasing and subsequently using. This principle has its origins in consumer protection. With the information that labels provide, consumers may make better, more informed choices about the products that they are thinking of buying. Furthermore, when products are properly labelled consumers can exercise their right to choose products that meet their particular economic,

5 A more detailed discussion of traceability, monitoring and labelling of GMOs can be found in Module 4 of this compendium.

health, religious, ethical, moral or other needs. For these reasons, labels can become a market-based mechanism that can contribute to the marketplace's acceptance of a product or the technology upon which the product is based.

A second aspect of labelling, related to the right to know, is protecting the consumer from false, misleading or deceptive practices. Labelling may be able to provide consumers enough information and to ensure that the claims made about a product are indeed true.

A third aspect of labelling is premised on consumer education. Consumer safety and environmental protection can be promoted when labels supply the appropriate information to consumers. For example, a label's information may warn the consumer of product attributes that could endanger his or her health or threaten the environment if the product is used in a certain way or is not kept or maintained adequately. In this way, labels can be viewed as a risk management tool.

When labels can or should be applied to products that may or not contain GMOs is a major issue that is being addressed at international and national levels. Labelling in the area of GMOs exists as both positive and negative information – that is, for claims that foods contain GMOs or that foods are GMO-free. Labelling can be voluntary or mandatory.

At the international level, the CPB sets out the obligations of parties concerning the identification of LMOs. Different obligations exist for LMOs intended for direct use as food or feed or for processing, LMOs destined for contained use and LMOs intended for intentional introduction into the environment (Art. 18). The TBT Agreement applies to all labelling requirements, including labelling of GMOs. The Codex Alimentarius Commission is preparing reference standards for the labelling of GMOs.

Table 3.1 | **Labelling requirements**

Examples of labelling requirements	Voluntary	Mandatory
GMO-free	Allowed for organic products in some jurisdictions such as the United States	No jurisdictions
Contains GMOs	All jurisdictions	European Union Transboundary movement of LMOs under CPB

LABELLING REQUIREMENTS
When labels can or should be applied to products that may or not contain GMOs is a major issue that is being addressed at international and national levels; different regulations in different jurisdictions exist.

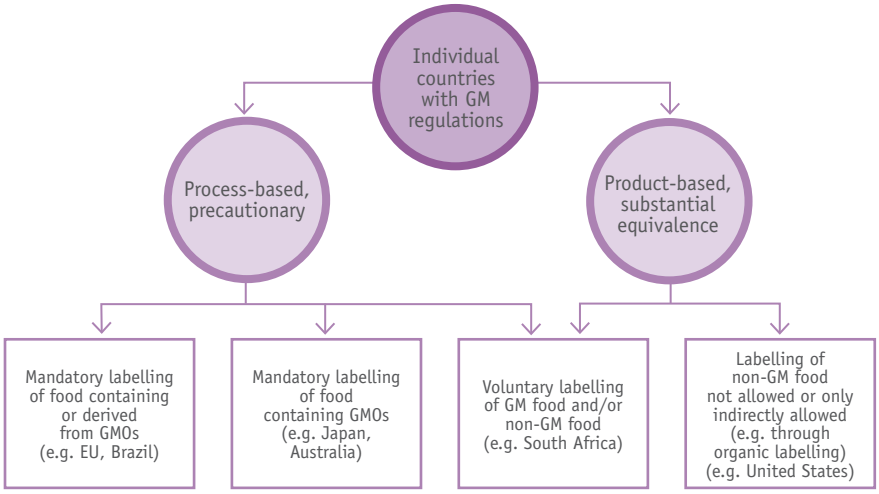
Labelling has been a particularly contentious area in international fora on biosafety. The main issues of contention return to the different risk approaches. Under strict theories of substantial equivalence with product-based approaches, there is no logical reason for requiring labels. States that hold this position fear that labelling requirements may be used as a protectionist measure to restrict trade.

Under process-based approaches, by contrast, and precautionary approaches that seek to accommodate uncertainty, labelling can be a public information and risk management tool. To date, these two approaches have been incompatible, although a majority of states have some labelling requirements, as required by the CPB, or in addition to CPB requirements.

Benefits of labelling can be summarized as protecting, informing, and educating consumers. Labelling can also serve as a compromise policy solution where political or regulatory consensus on risk regulation is not possible. Drawbacks include additional costs to producers and manufacturers, which will likely be passed on in turn to consumers. These costs arise from the requirement that labelling be accurate and useful, which in turn necessitates effective segregation, traceability and monitoring systems.

CLASSIFICATION OF LABELLING REGULATIONS
Regulations can be mainly grouped into process-based versus product-based approaches and according to the resulting mandatory/ voluntary labelling requirements.

Figure 3.1 | **Classification of labelling regulations**
(*Adapted from*: Gruère, 2006)



SEGREGATION, IDENTITY PRESERVATION, AND TRACEABILITY
Ensure that there is no unintentional admixture of GMO and non-GMO products that could lead to unintentional releases of LMOs or adventitious presence of GMOs in food, feed or processed products.

3.12 SEGREGATION, IDENTITY PRESERVATION, AND TRACEABILITY

Segregation, identity preservation and traceability ensure that there is no unintentional admixture of GMO and non-GMO products that could lead to unintentional releases of LMOs or adventitious presence of GMOs in food, feed or processed products. They are also critical elements of any effective labelling regime.

Segregation or ensuring that GMO and non-GMO products are kept separate and that there is no unintentional admixture, can be achieved by either specializing in biotech or non-biotech (both on the farm and the subsequent processing steps), establishing separate facilities for biotech and non-biotech, or taking precautions to separate biotech and non-biotech production (including a thorough

cleaning of equipment and storage facilities after each biotech variety). As an alternative to segregation, processors can choose to reformulate their products to use ingredients from crops that are exclusively non-biotech, thus minimizing the risk of inadvertently using a biotech variety.

The cost of any of these options varies greatly depending on the flexibility of the production and marketing systems, the tolerance level for biotech content, the volume of biotech and non-biotech commodities and products processed by the system, and the likelihood of achieving economies of scale.

Another set of costs arises in convincing manufacturers and consumers that the product is truly non-biotech. One way to achieve this is to test for biotech content, and a number of private firms have begun to market biotech-testing products.

Another method of monitoring the integrity of the non-biotech label is to establish a system of IP for both GMO and non-GMO products (see Box 3.5) in which producers track each stage of the marketing chain and can thus attest to the integrity of their non-biotech products. Such a system relies on strict segregation and product tracking more than on continual testing.

The costs of non-compliance can also be high, as is evident from the case of adventitious presence of non-approved GMO rice in commercial rice exports (see Box 3.7).

In addition, it may be difficult for individual firms and farmers to establish a credible non-biotech label. Consumers may be sceptical of producers' claims. Such scepticism could be fuelled by the observation that biotech tests are not completely reliable or consistent, and that it is difficult to ensure the integrity of an IP system. To this end, standards, traceability, testing, certification and enforcement could all facilitate the development of a market for non-biotech foods.

BOX 3.6

IDENTITY PRESERVATION (IP)

Identity preservation (IP) is an important measure for traceability: every product which is a genetically modified organism, or which contains genetically modified ingredients, must be accompanied by documents detailing the identity of this GMO during the whole production chain. For this purpose, the OECD introduced a naming system called Unique Identifiers. Should a GMO have to be withdrawn from the market, IP allows authorities to trace all shipments up to the food stores (emphasis in original)

From: EU Co-extra glossary, http://www.coextra.eu/glossary/word694.html

IDENTITY PRESERVATION
Identity preservation is an important measure for traceability: every product which is a genetically modified organism, or which contains genetically modified ingredients, must be accompanied by documents detailing the identity of this GMO during the whole production chain.

Biotech standards or tolerance levels would determine the maximum amount of biotech ingredients allowable in a "non-biotech" commodity or food. Consistent enforcement of standards, testing and certification would also decrease transaction costs and increase market efficiency. This, along with added public trust, makes it an important policy goal.

3.13 ISSUES OF INTEREST FOR COUNTRIES THAT HAVE NOT YET ADOPTED BIOSAFETY LEGISLATION

ISSUES OF INTEREST FOR COUNTRIES THAT HAVE NOT YET ADOPTED BIOSAFETY LEGISLATION
Countries that have not yet adopted biosafety regulations must take into consideration the above-mentioned international agreements.

Countries that have not yet adopted biosafety regulations must take into consideration the above-mentioned international agreements. Import regulations in particular will require compliance with the standards of any international agreements to which the country is (or hopes to become) a signatory. Additionally, a country's regulations on issues such as notification, commercial approvals, identity preservation, traceability, labelling and monitoring may affect its ability to export to countries with different requirements.

BOX 3.7

UNAPPROVED RICE CONTAMINATION EVENTS

An example of how the issues of identity preservation, traceability and monitoring arise in international trade comes from the 2006-2007 case of adventitious presence of unapproved varieties of genetically modified rice in rice crops in the southern United States.

The unapproved variety was found in commercial rice seeds and entered the food and feed system in the United States. While the USDA later determined that the genetically modified variety posed no safety concerns for food or feed use, and subsequently granted it approval for commercialization, the contamination incidents had broad-ranging and serious trade effects.

The genetically modified rice was also found in rice imports from the United States to a number of countries in Europe, the Middle East and Asia (another unapproved genetically modified variety was also discovered in rice exported from China).

After the discovery of the adventitious presence of Bayer LL Rice 601, Japan suspended imports of long-grain rice from the United States. The European Commission adopted a decision banning all consignments of United States long-grain rice except those tested by an accredited laboratory and certified as free from the genetically modified variety.

United States rice farmers have filed several lawsuits against USDA and Bayer for losses due to the contamination; there are claims of up to USD 1.2 billion in losses due to lost exports and closed markets. The litigation, as of July 2010, is still ongoing, but juries have already held Bayer liable for over USD 50 million to compensate farmers for their losses, and is indicative of the high stakes involved in ensuring compliance with biosafety regulations.

The case also shows the importance of meeting IP, traceability and containment standards. It demonstrates the role of testing and monitoring in ensuring a successful trade regime, especially in an environment where different countries may have different import standards and requirements.

UNAPPROVED RICE CONTAMINATION EVENTS
An example of how the issues of identity preservation, traceability, and monitoring arise in international trade comes from the 2006-2007 case of adventitious presence of unapproved varieties of genetically modified rice in rice crops in the southern United States.

Most developing countries do not export GMOs, but many do export conventional products, and therefore "…find themselves in a particularly difficult situation: in order to preserve their export opportunities, especially towards markets that are skeptical about bioengineered products, they may need to be 'GM-free' countries. This means not only that they should not be exporters of GMOs, but also that they should not be producers of GMOs for domestic consumption and not even importers of GMOs. Losing 'GM-free' status is perceived by some countries as having negative repercussions for their export opportunities for all agricultural products" (Zarrilli, 2005). This perception has the potential to limit choice for developing countries. At the same time, developing countries may feel pressured by GMO-exporting countries to make regulatory decisions based on the ideas of substantial equivalence and "product, not process."

As Zarrilli (2005) writes, "While developed countries have established their national frameworks… focusing primarily on domestic priorities and strategies, most developing countries are doing so under less flexible circumstances…. …[D]eveloping countries increasingly seem to be expected to set up their national regulatory schemes based on the requests and expectations of their main trade partners."

Indeed, all countries need to address both constraints, in terms of requirements of international agreements to which they or their trading partners are signatories, and expectations, in terms of goals and legal frameworks of their trading partners. While several issues remain open and unresolved, international agreements generally seek to harmonize and streamline regulations and requirements.

3.14 CONCLUSIONS: CHAPTER 3

Implementation of (national) biosafety legal instruments involves the establishment of appropriate mechanisms for implementing international agreements, conducting risk analysis, including public participation, notifying trading partners and the public, and ensuring compliance through monitoring, management, and mechanisms for addressing non-compliance.

Other concerns to address include opportunities for international cooperation at a technical level (sharing human and scientific resources and expertise), establishing a scheduled phasing-in of regulations (for example, initial voluntary guidelines entrenched in legislation over time), and creating a means for revising the framework in response to new data and/or requirements of international agreements.

CONCLUSIONS: CHAPTER 3
Implementation of (national) biosafety legal instruments involves the establishment of appropriate mechanisms for implementing international agreements, conducting risk analysis, including public participation, notifying trading partners and the public, and ensuring compliance through monitoring, management and mechanisms for addressing non–compliance.

ANNEX 1

INTERNATIONAL LEGAL INSTRUMENTS ADDRESSING BIOTECHNOLOGY AND BIOSAFETY

Year	Treaty, agreement, or law	Decision-making body	Parties (as of January 2010)	Focus	Binding	Direct or indirect application to biotechnology	Web site
1963	Codex Alimentarius	WHO, FAO	183	Food safety	No	Direct	http://www.codexalimentarius.net/web/index_en.jsp
1982	Convention on Law of the Sea	UN	157	Fisheries and oceans	Yes	Indirect	http://www.un.org/Depts/los/convention_agreements/convention_overview_convention.htm
1985	Guidelines for Consumer Protection	UN		Consumer protection	No	Indirect	http://www.un.org/esa/sustdev/publications/consumption_en.pdf
1987	World Conservation Union (IUCN) Position Statement on Translocation of Living Organisms	IUCN	Government and NGO Members at various levels	Biosafety	No	Direct	http://www.iucnsscrsg.org/download/IUCNPositionStatement.pdf
1991	Code of Conduct for the Release of Organisms into the Environment	UNIDO		Biosafety	No	Direct	http://www.biosafety.gov.cn/image20010518/5079.pdf
1992	Agenda 21, Chapter 16	UN	Over 178 signatories	Sustainable development, "Environmentally sound management of biotechnology"	No	Direct	http://www.unep.org/Documents.Multilingual/Default.asp?DocumentID=52&ArticleID=64
1992	Convention on Biological Diversity	CBD	193	Biosafety	Yes	Direct	http://www.cbd.int/convention/convention.shtml
1992	Safety Considerations for Biotechnology	OECD		Biosafety	No	Direct	http://dbtbiosafety.nic.in/guideline/OACD/Safety_Considerations_for_Biotechnology_1992.pdf
1993 (reviewed 2001, 2004, 2006)	Code of Conduct on Plant Biotechnology as it Relates to Plant Genetic Resources for Food and Agriculture	FAO		Biosafety	No	Direct	ftp://ftp.fao.org/ag/cgrfa/cgrfa9/r9w18ae.pdf

Year	Treaty, agreement, or law	Decision-making body	Parties (as of January 2010)	Focus	Binding	Direct or indirect application to biotechnology	Web site
1994	Agreement on Application of Sanitary and Phytosanitary Measures (SPS)	WTO	153 WTO Members	Trade and human health	Yes	Direct	http://www.wto.org/english/tratop_e/sps_e/sps_e.htm
1994	Agreement on Technical Barriers to Trade (TBT)	WTO	153 WTO Members	Trade	Yes	Direct	http://www.wto.org/english/tratop_e/tbt_e/tbt_e.htm
1994	Trade Related Aspects of Intellectual Property Rights (TRIPs)	WTO	153 WTO Members	Trade and IPRs	Yes	Indirect	http://www.wto.org/english/tratop_e/trips_e/trips_e.htm
1995	Code of Conduct on Responsible Fisheries	FAO		Fisheries	No	Indirect	http://www.fao.org/DOCREP/005/v9878e/v9878e00.htm
1995	Technical Guidelines on Biosafety	UNEP	58 Members chosen from UN General Assembly Members	Biosafety	No	Direct	http://www.unep.org/biosafety/Documents/Techguidelines.pdf
1996	Code of Conduct for the Import and Release of Exotic Biological Control Agents	FAO		Biosafety, biocontrol	No	Indirect	http://www.fao.org/docrep/x5585E/x5585e0i.htm
1997	International Plant Protection Convention (IPPC)	IPPC/ FAO	172 Members	Biodiversity, agriculture, biosafety	Yes	Direct	https://www.ippc.int/servlet/CDSServlet?status=ND0xMzI5MiY2PWVuJjMzPSomMzc9a29z
1998 with 2005 (addendum on GMOs)	Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (Aarhus Convention)	UNECE	44	Public participation, democracy, environmental rights, human rights	Yes	Direct	http://www.unece.org/env/pp/treatytext.htm
2000	Cartagena Protocol on Biosafety	CPB	157	Biosafety	Yes	Direct	http://www.cbd.int/biosafety/
2004	International Treaty on Plant Genetic Resources for Food and Agriculture	FAO	120	Biosafety, Agriculture	Yes	Indirect	http://www.planttreaty.org/

ANNEX 2

AREAS COVERED BY INTERNATIONAL TREATIES THAT INCLUDE PROVISIONS ON BIOSAFETY

International agreement	Environmental health/biodiversity	Human health	Trade	IPR	Access and benefit-sharing (for genetic resources)	Liability	Consumer protection/ information	Participation and democracy	Standard-setting
Codex Alimentarius		x	x				x		x
IPPC	x		x						x
CBD	x	x	x		x			x	
Cartagena Protocol	x	x	x		x			x	
SPS	y	x	x						y
TBT	y	y	x		y				y
TRIPS			x	x	y				
Law of the Sea	x		x						
Aarhus Convention	y	y	y		y		x	x	
ITPGRFA	x		x	x	x				

X = directly concerned with; Y = interacts with or indirectly affects

REFERENCES

Antonovics, J. 1999. Pathogens and plant population dynamics: The effects of resistance genes on numbers and distribution, pages 57-64. Plenary Paper, Proceedings of a Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems, eds. Traynor, Patricia L. and James H. Westwood. Information Systems for Biotechnology. Blacksburg, VA. pages 49-55.

Aust, A. 2000. *Modern Treaty Law and Practice*. Cambridge, Cambridge University Press.

EU. Co-extra Glossary (available at: http://www.coextra.eu/glossary/word694.html)

Gaugitsch, H. 2002. Experience with environmental issues in GM crop production and the likely future scenarios. *Toxicology Letters* 127: 351-357.

Gerdung, A. 2006. Germany's Liability Law for GMO Cultivation, 37. Wellington, Sustainability Council of New Zealand (Available at: http://www.sustainabilitynz.org/docs/GermanLiabilityLawforGMCultivation.pdf)

Gerstetter, C., Görlach, B., Neumann, K. & Schaffrin, D. 2007. The International Treaty on Plant Genetic Resources for Food and Agriculture within the current legal regime complex on plant genetic resources. *The Journal of World Intellectual Property* 10(3/4): 259-283.

Glowka, L. 2003. Law and modern biotechnology: selected issues of relevance to food and agriculture, 172. Rome, FAO.

Gruère, G. P. 2006. A preliminary comparison of the retail level effects of genetically modified food labelling policies in Canada and France. *Food Policy* 31: 148-161.

Gruère, G. & Rao, S.R. 2007. A review of international labelling policies of genetically modified food to evaluate India's proposed rule. *AgBioForum* 10(1) art. 6.

Hallerman, E.M. & Kapuscinski, A.R. 1995. Incorporating risk assessment and risk management into public policies on genetically modified finfish and shellfish. *Aquaculture* 137: 9–17.

Hannam, I. & Boer, B. 2002. Legal and institutional frameworks for sustainable soils: a preliminary report. IUCN Environmental Policy and Law Paper No. 45. IUCN Environmental Law Programme, IUCN.

Hautea, D.M. 2008. Lecture Module 3: Risk Analysis for GMOs: Concepts, Methods and Issues. Project TCP/BGD/3102. FAO Biosafety Training Workshop, 20-30 November 2008, Dhaka, Bangladesh.

Holmes, P. 2006. Trade and 'domestic' policies: the European mix. *Journal of European Politics* 13(6): 815-831 (available at: http://www.wto.org/english/tratop_e/dispu_e/cases_e/ds26_e.htm)

ISPM (International Standards for Phytosanitary Measures) No. 11. 2004. *Pest risk analysis for quarantine pests including analysis of environmental risks and LMOs.* Available at https://www.ippc.int/servlet/BinaryDownloaderServlet/34163_ISPM_11_E.pdf?filename=1146658377367_ISPM11.pdf&refID=34163

Kareiva, P. & Marvier, M. 2000. Case Study 3: Genetically Modified Organisms: An overview of risk assessment applied to genetically modified crops, pages 231-246. *In* Incorporating Science, Economics, and Sociology in Developing Sanitary and Phytosanitary Standards in International Trade: Proceedings of a Conference. National Research Council. Washington DC, National Academies Press.

Mackenzie, R., Burhenne-Guilmin, F., La Viña, A.G.M. & Werksman, J.D. in cooperation with A. Ascencio, J. Kinderlerer, K. Kummer, and R. Tapper. 2003. An explanatory guide to the Cartagena Protocol on Biosafety. IUCN Environmental Policy and Law Paper No. 46. Gland: IUCN Environmental Law Centre, IUCN.

Majone, G. 2002. What price safety? The precautionary principle and its policy omplications. *JCMS* 40(1): 89-109.

Marvier, M. & Kareiva, P. 1999. Extrapolating from field experiments that remove herbivores to population-level effects of herbivore resistance transgenes, pages 57-64. Plenary Paper, Proceedings of a Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems, eds. Traynor, Patricia L. and James H. Westwood. Information Systems for Biotechnology. Blacksburg, VA..

McLean, M. A., Frederick, R.J., Traynor, P.L., Cohen, J.I. & Komen, J. 2002. A conceptual framework for implementing biosafety: linking policy, capacity, and regulation. Briefing Paper No. 47. The Netherlands, ISNAR.

Oberthür, S. & Gehring, T. 2006. Institutional interaction in global environmental governance: the case of the Cartagena Protocol and the World Trade Organization. *Global Environmental Politics* 6(2): 1-31.

OIE. 2008: Report of the Meeting of the OIE Biological Standards Commission. Paris (available at http://www.oie.int/downld/SC/2008/A_BSC_sept2008.pdf).

Peel, J. 2006. A GMO by any other name ... might be an SPS risk!: implications of expanding the scope of the WTO Sanitary and Phytosanitary Measures Agreement. *European Journal of International Law* 17: 1009-1031.

Ravi, B. From the training material produced for the Biosafety Training held in Bangladesh in November 2008.

The Royal Society. 2002. Genetically modified plants for food use and human health – an update. Policy Document 4/02. The Royal Society Online (available at: http://www.royalsoc.ac.uk;).

Sarquis, A. 2004. El comercio de commodities y el Protocolo de Cartagena. Subsecretaría de Política Agropecuria y Alimentos, Dirección Nacional de Mercados Agroalimentarios, Secretaría de Agricultura, Ganadería, Pesca y Alimentos, Argentina.

Spreij, M. 2007. The SPS Agreement and Biosafety. FAO Legal Papers Online No. 65. Rome, FAO.

Zarrilli, S. 2005. International trade in GMOs and GM products: national and multilateral legal frameworks. Policy Issues in International Trade and Commodities Study Series No. 29. New York, UNCTAD.

UNEP. 2007. Glossary of Terms for Negotiators of Multilateral Agreements.

USEFUL READINGS

Cohen, J.I. 2001. Harnessing biotechnology for the poor: challenges ahead for capacity, safety and public investment. *Journal of Human Development* 2(2): 239-263.

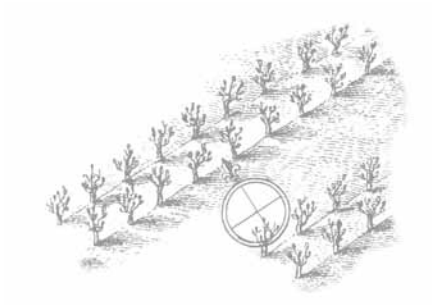
Klapper, B.S. 2006. WTO rules against EU modified food ban. *Associated Press* 21 November.

Kysar, D. 2004. Preferences for processes: the process/product distinction and the regulation of consumer choice. *Harvard Law Review* 118: 525-642.

Nap, J-P., Metz, P.L.J., Escaler, M. & Conner, A.J. 2003. The release of genetically modified crops into the environment: Part I. Overview of current status and regulations. *The Plant Journal* 33: 1-18.

National Research Council Committee on Scientific Evaluation of the Introduction of Genetically Modified Microorgansims and Plants into the Environment. 1989. *Field testing Genetically Modified Organisms: framework for decisions.* National Academy of Sciences.

Schauzu, M. 2000. The concept of substantial equivalence in safety assessment of foods derived from genetically modified organisms. *AgBiotechNet* 2 (ABN 044): 1-4.



Design and layout: Pietro Bartoleschi and Arianna Guida (Studio Bartoleschi)
Cover illustrations elaborated from “l’Encyclopédie Diderot et d’Alembert”
Printed in Italy on ecological paper, Forest Stewardship Council (FSC) certified, May 2011

Biosafety Resource Book

MODULE E

LEGAL ASPECTS

provides an overview of the existing legal tools and frameworks on biotechnology and biosafety, and offers a thorough description of the international instruments that regulate biosafety and their interactions.

For additional information please consult
www.fao.org/biotech
or contact
biotech-admin@fao.org

Biosafety Resource Book

a INTRODUCTION TO MOLECULAR BIOLOGY AND GENETIC ENGINEERING

b ECOLOGICAL ASPECTS

c RISK ANALYSIS

d TEST AND POST-RELEASE MONITORING OF GENETICALLY MODIFIED ORGANISMS (GMOs)

e LEGAL ASPECTS

This Biosafety Resource Book stems from the experience gained in biosafety capacity development projects and is based on the materials developed by the lecturers who have taught in the training courses organized to date.

The Resource Book has been prepared in response to an expressed need, with the purpose of being used as a training tool in future activities. The Resource Book also aims at providing biosafety regulators, policy makers and members of the national biosafety committees with reference materials that can be readily consulted beyond the training events, when the need arises.

For additional information please consult
www.fao.org/biotech
or contact
biotech-admin@fao.org

ISBN 978-92-5-106718-5

I1905E/1/01.11